GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND
VARIATION

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VARIATION

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CORRIGENDA

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Page 93, line 1 in Literature Cited, for "ANDERSON, E. C." read "ANDERSON, E. G."

Page 371, line 5, for "numbers of young" read "numbers of F_2 young."

Page 379, line 2, for "*HrHr*" read "*Hrhr*."

Page 396, line 11, for "monohybrid ratio for pollen color when selfed or when crossed" read "monohybrid ratio for pollen color when crossed."

Page 403, Table 1, line 4 under "Green," for "Pale Turtle Green . . . d" read "Pale Turtle Green.....f."

Page 438, Table 4, under Plant Number column, lines 1 in upper and lower halves, for "2434–9" read "2464–9."

Page 438, Table 4, under Plant Number column, line 6 in upper half, delete "2464-4.*"

Page 446, line 7, for "pollen or trisomics" read "pollen of trisomics."

PIERRE LOUIS FRANCOIS LÉVÊQUE DE VILMORIN

(FRONTISPIECE)

A biographical sketch of any one member of the VILMORIN family would be only an incomplete and isolated part of the whole, unless woven into the very fascinating and unique history of the family and firm of VILMORIN ANDRIEUX ET CIE, famous among seedsmen and plant breeders of the world since 1774, when PHILIPPE VICTOIRE LÉVÊQUE DE VILMORIN married Mademoiselle ADELAIDE D'ANDRIEUX, daughter of M. ANDRIEUX, who had a seed and plant shop on the present site of the head offices of the firm in Paris, Quais de la Megisserie, called at that time Quai de la Ferraille.

PHILIPPE VICTOIRE LÉVÊQUE DE VILMORIN was an educated gentleman of Lorraine origin. He became the partner of his father-in-law under the firm name ANDRIEUX ET VILMORIN. Soon after the death of M. ANDRIEUX in 1779, the firm name was changed to its present form. This first of the VILMORIN seedsmen was a great lover of plants. He became acquainted with the leading botanists of his time and did much to improve and distribute desirable plants, trees, shrubs and bulbs. He was one of the first in France to publish catalogs of seeds and plants, with detailed instructions on methods of cultivation. This pioneer member of the VILMORIN seed firm died in 1804, leaving the business to his eldest son, PHILLIPPE ANDRÉ LÉVÊQUE DE VILMORIN, who was already a partner in the firm. This scion of the VILMORIN stock took a keen interest in the improvement of cereals, forage plants, trees and shrubs. He soon engaged in scientific and experimental work, and took partners to assist in the business of the firm. He acquired land at Verrieres le Buisson for herbaceous plants, and at Les Barres, Loiret, for trees. The VILMORIN establishment at Verrieres is today one of the largest and finest in the world, for the improvement of plants and the production of seed stocks. The place at Les Barres is now a government forestry school. In 1815, the potato collection of PARMENTIER was given to VILMORIN by the Societé Royal d'Agriculture, and the collection has since been grown at Verrieres. This second member of the famous line of VILMORINS retired from active business in 1843 and died in 1862, leaving the management of the firm to PIERRE LOUIS FRANCOIS LÉVÊQUE DE VILMORIN (April 16, 1816–March 21, 1860), the particular subject of this sketch.

This VILMORIN III, as we may designate him, was only 27 years old when he became head of the firm. He made systematic studies of heredity in plants, and used the method of individual selection, growing the progeny of each selected plant, separately. He put this method of individual selection, originated by his father, into practical use, and by it produced the Vilmorin strain of sugar beet, but little surpassed in sugar content, today.

The form of the first roots was not perfect, but the new types he obtained very soon after 1850 gave a regular tap-rooted plant. LOUIS DE VILMORIN began by selecting roots on the basis of density, taking a small cylinder of flesh from the root and testing its density in sugar and afterwards in salt solutions of different concentrations. Both these methods had drawbacks. After 1852 DE VILMORIN determined the density by means of a silver ingot, and in 1853 he mentions analyses made by himself with the Rotatametre Biot and Saccharimetre Soleil. He published the results of his important work at the Academie des Sciences in 1856.

The wife of LOUIS DE VILMORIN, Madame ELISA L. DE VILMORIN, assisted him in his experiments and on the trial grounds. She published a monograph on strawberries in DECAISNE'S *Jardin fruitier du Museum*. LOUIS VILMORIN died in 1860 at 44 years of age, leaving three sons.

The eldest son, CHARLES HENRY LOUIS L. DE VILMORIN, born in 1843, became associated with his mother in the business, and in 1873 became head of the firm, associated with his brother, MAURICE. The third son, PHILIPPE, was killed at Le Mans on the last day of the Franco-Prussian war of 1870–1871.

This VILMORIN IV, as we may call him, carried on still more extensively the plant breeding experiments of his father and grandfather. He produced many improved varieties of cultivated plants by crossing and selection, among them, several vegetables and garden flowers, sugar beets and wheat. He classified potatoes and published the first edition of the "*Catalogue methodique et synonymique des Pommes de terre.*" He published two editions of "*Catalogue des Froments*" and also "*Les Meilleurs Bles.*" He also revised and enlarged "*Les plants potagiers,*" and published a number of other papers on scientific and practical subjects. This fourth in succession in the line of VILMORINS died in 1899, leaving five sons and two daughters. His wife, Madame HENRY L. DE VILMORIN, lived to be past eighty years old, enjoying the summer seasons at the delightful Verrieres estate and the winter months in the Riviera.

MAURICE L. DE VILMORIN, younger brother of HENRY L. DE VILMORIN and junior member of the firm, maintained a country home at Les Barres, Loiret, where he devoted himself to studies of forest and ornamental trees. Close to the arboretum of his grandfather, he established the largest collection of shrubs in France, of which he published the catalog in 1904, under the title, "*Fructicetum Vilmorianum.*" Up to his death in 1918, he maintained close relations with missionaries in China, and received from them seeds of several thousand species, mostly woody plants.

J. M. PHILIPPE LÉVÊQUE DE VILMORIN, the eldest son of HENRY, born in 1872, entered the firm while still young and became head of it in 1899, after the death of his father. In accordance with the family tradition, this VILMORIN V, as we may call him, made careful preparation for his duties, by studying botany and related scientific subjects. He enlarged the family collections of herbaceous and woody plants, built a rockery and established laboratories for research in chemistry, biology and plant pathology. He carried on plant breeding experiments with cereals and potatoes and produced improved varieties of sweet peas and turf grasses. When Mendel's law was rediscovered in 1900, he devoted himself to its study, using peas and wheat as his experimental plants. He became the first geneticist in France and was a leading figure in the Genetics Congress held in Paris in 1911.

In 1900, PHILIPPE LÉVÊQUE DE VILMORIN married Mademoiselle MELANIE DE DORTAN, who came of an old French family having a large estate at Dompierre les Ormes, Saone et Loire. VILMORIN started an arboretum on one of the farms, and covered about fifty acres with species adapted to the severe soil and climatic conditions of that hilly region.

Many English and American seedsmen and plant breeders with whom VILMORIN had intimate contacts mourned at his death, March 1, 1926. Madame PHILIPPE L. DE VILMORIN took a very deep interest in the management of the firm after the death of her husband. She aided in maintaining the plant collections and the arboretum, made additions to the library, and helped in entertaining distinguished foreign visitors.

M. JACQUES L. DE VILMORIN, here referred to as VILMORIN VI, eldest son of M. MAURICE, entered the firm some years before the death of his father. He

became general manager of the business in Paris and also director of the experimental and scientific work at Verrieres. He was a leading world authority on the sugar beet, and published his Sorbonne doctorate thesis, "*L'heredite chez la Betterave cultivée*," in 1925. He and his associates have published several papers on wheat, with special reference to quality, its testing and improvement. He was a member of the Academie d'Agriculture, and won the Legion of Honor. He had a host of friends on the continent, in the British Isles and in America, who mourned his untimely death in 1933.

M. HENRY LOUIS DE VILMORIN, younger brother of PHILIPPE, entered the firm after the World War. Like his distinguished ancestors, he interested himself not only in the business, but also in scientific work and took for his special field, the improvement of potatoes. He is well-known among Scotch and English potato experts and growers, and has taken an active interest in the potato variety trials and classification studies at Ormskirk, England.

M. ROGER DE VILMORIN, son of M. and Madame PHILIPPE, gained his science licentiate in 1927, and since then has had varied experience in several business and technical departments of the firm. His research interest is plant cytology, in which field he has published several excellent papers. ROGER DE VILMORIN attended the Sixth International Genetics Congress held at Ithaca, N. Y. in 1932, and endeared himself to all who came to know him there, because of his modesty, personal charm and obvious genuine interest in science.

A bibliography of the publications of five generations of the VILMORIN family comprises numerous trade, technical and scientific publications, and occupies more than thirty closely typed pages.

In honoring PIERRE LOUIS FRANCOIS LÉVÊQUE DE VILMORIN, GENETICS joins with seedsmen, plant breeders and geneticists in all parts of the world in paying homage to the whole VILMORIN family, and in wishing the firm of VILMORIN ANDRIEUX ET CIE continued success and richly deserved prosperity.

In closing, it is appropriate to quote an inscription from a monument to VILMORIN, "Without the Vilmorins, there would have been lower yields of wheat in our fields, less sugar in our sugar beets, and fewer flowers in our gardens."

The portrait here reproduced was re-engraved from a copper half-tone furnished by the family. This biographical sketch has been prepared by Dr. JOHN H. PARKER of the KANSAS STATE COLLEGE, Manhattan, Kansas.

STUDIES IN HUMAN INHERITANCE. X.

A TABLE TO DETERMINE THE PROPORTION OF RECESSIVES TO BE EXPECTED IN VARIOUS MATINGS INVOLVING A UNIT CHARACTER*

LAURENCE H. SNYDER
Ohio State University, Columbus, Ohio

Received February 9, 1933

In genetic studies on animals and plants, the proof of the allelomorphic nature of two genes, one of which is dominant, has depended largely on the obtaining of the 3:1 ratio from the matings of known heterozygotes or of the 1:1 ratio from the backcross. Heterozygotes have been relatively easy to determine in such cases by using F_1 individuals, or by making test matings. When we approach the study of human heredity, however, we are unable to make test matings, and we are faced with considerable difficulty in determining whether an individual exhibiting a dominant trait is homozygous or heterozygous. In some instances this may be determined from a knowledge of the individual's parents or offspring. In many cases, however, our data are limited to two generations. Furthermore, a human family is never large enough to prove that an individual showing a dominant trait and producing no recessive offspring is really homozygous.

It becomes necessary, therefore, in the study of human heredity, to use an analysis which obviates the need of distinguishing between homozygous and heterozygous dominants. In a random series of matings where both parents show a dominant character, the offspring will show dominance and recessiveness, respectively, in a proportion greater than 3:1, because of the fact that some of the dominant parents are homozygous, and will produce all dominant offspring. Similarly, in a random series of matings in which one parent shows the dominant character, the other the recessive, the ratio of dominance to recessiveness among the offspring will be greater than 1:1.

The exact proportions of recessives to be expected in these types of matings may, however, be predicted very exactly by an analysis of the genes on a frequency basis. These proportions will vary among various factors, depending on the relative frequencies of the allelomorphic genes in the population, and will vary for the same factor among different races, if the frequencies of the genes vary from race to race.

The expected proportions of recessive offspring from the matings of

* Part of the cost of the mathematical composition and the tables in the article is paid by the GALTON AND MENDEL MEMORIAL FUND.

dominants with dominants, and dominants with recessives, respectively, are calculated as follows:

Let p = frequency of the dominant gene of a pair of allelomorphs, and let q = frequency of the recessive gene.

Then $p+q=1$. It is readily seen that

p^2+2pq = the dominant individuals of the population. (A)

q^2 = the recessive individuals of the population. (B)

$q=\sqrt{B}$

$p=1-\sqrt{B}$

A relationship may be readily demonstrated between the frequencies of the allelomorphic genes and the proportions of recessive offspring to be expected in random matings of dominants with dominants, and dominants with recessives.

Since p^2 = homozygous dominants and $2pq$ = heterozygous dominants,

$$\frac{p^2}{p^2+2pq} = \text{proportion of dominants which are homozygous}$$

$$\frac{2pq}{p^2+2pq} = \text{proportion of dominants which are heterozygous}$$

The only recessive offspring produced in matings of dominants with dominants will be one-quarter of the offspring of matings of heterozygous dominants with heterozygous dominants. Similarly, the only recessive offspring produced in matings of dominants with recessives will be one-half of the offspring of the matings of heterozygous dominants with recessives. Formulae for these proportions are derived as follows:

Let R = proportion of recessive offspring to be expected from matings of dominants with dominants,

And S = proportion of recessive offspring to be expected from matings of dominants with recessives.

Then $$R=1/4\left(\frac{2pq}{p^2+2pq}\right)^2=\left(\frac{q}{p+2q}\right)^2$$

and $$S=1/2\left(\frac{2pq}{p^2+2pq}\right)=\frac{q}{p+2q}.$$

Since the results of these formulae will vary depending on the frequencies of the genes concerned, which in turn are derived from the proportion of recessive individuals in the general population, the following table has been prepared. This table gives directly from the observed proportion of recessive individuals in the population, the expected proportion of recessive offspring from matings of dominants with dominants, and dominants

with recessives. The calculations were done to six decimal places with an electric Marchant calculator, and the results are presented in the table to the nearest fourth decimal place.

While the table will be particularly useful in studies of human heredity, it will be found applicable to studies of animals and plants, where it is impractical or impossible to determine the heterozygosity of dominants. It is not necessary, for example, to make the general statement so often encountered in genetic studies, that "from certain matings of dominant with dominant, a ratio approaching 3:1 was obtained."

As an example of the use of the table, it is found that among white Americans, .298 are unable to taste phenyl-thio-carbamide (SNYDER 1932, Ohio Journal of Science 32:436). This taste deficiency appears on simple inspection of the family histories, to be an autosomal recessive character. To further prove the unit character nature of the deficiency, the table indicates that in matings of tasters with tasters (Column R) we should expect .1247 of the offspring to be recessive, that is, taste-deficient. From a study of 800 families we find that in such matings .1228 of the offspring are recessive, a difference of $.0019 \pm .007$. Similarly, in matings of tasters with non-tasters, we find (Column S), that we should expect .3531 of the offspring to be recessive if we are really dealing with a unit character. The proportion actually found is .3653, a difference of $.0122 \pm .012$.

The probable errors of the observed and calculated proportions are as follows:

For the P.E. of the observed proportions, the well-known formula $.6745\sqrt{\frac{\overline{xy}}{n}}$ is used, where x = observed proportion of dominant children, y = observed proportion of recessive children, and n = total number of offspring, for each type of mating.

For the calculated proportions, where N = 100 or more, the formulae are

$$\text{P.E.}\left(\frac{q}{p+2q}\right)^2 = \frac{.6745\,(1-\sqrt{b})}{(1+\sqrt{b})^2}\sqrt{\frac{b}{N(1-b)}}$$

$$\text{P.E.}\quad \frac{q}{p+2q} = \frac{.6745\,(1-\sqrt{b})}{2(1+\sqrt{b})}\sqrt{\frac{1}{N(1-b)}}$$

where N = Total number of individuals tested in deriving A and B. In practice, b, which is the true value of the proportion of recessives in the population, must be taken as B, the observed value from the complete sample of individuals studied, just as in the foregoing familiar formula $.6745\sqrt{\frac{\overline{xy}}{n}}$, x and y actually represent the true proportions, but are in practice taken as the observed proportions.

Since p and q are subject to error because they are derived from A and B, which are themselves representative only of a sample of the whole population, the derivation of these formulae is as follows:

DERIVATION OF A FORMULA FOR THE PROBABLE ERROR OF $\frac{q}{p+2q}$

Let b = true value of the proportion of recessives in the population.
And B = the value of b obtained from a sample of the population.
Then $B = b+e$
In which e = the difference between the value of b calculated from the sample and the true value of b.
Since True $q = \sqrt{b}$
and True $p = 1-\sqrt{b}$

Then True $\frac{q}{p+2q} = \frac{\sqrt{b}}{1+\sqrt{b}}$

But the value of b calculated from a sample may be in error by an amount e so that the obtained value of $q = \sqrt{b+e}$

Hence calculated $\frac{q}{p+2q}$ may be in error by an amount equal to Calculated $\frac{q}{p+2q}$ − True $\frac{q}{p+2q}$ or E, the error in $\frac{q}{p+2q} = \frac{\sqrt{b+e}}{1+\sqrt{b+e}} - \frac{\sqrt{b}}{1+\sqrt{b}}$ (1)

Hence, $$E = \frac{1}{1+\sqrt{b}} - \frac{1}{1+\sqrt{b+e}} \tag{2}$$

$$\frac{1}{1+\sqrt{b+e}} = \frac{1}{1+\sqrt{b}} - E \text{ or } \frac{1}{1+\sqrt{b+e}} = \frac{1-(1+\sqrt{b})E}{1+\sqrt{b}}$$

Inverting, $$1+\sqrt{b+e} = \frac{1+\sqrt{b}}{1-(1+\sqrt{b})E} \text{ or } \sqrt{b+e} = \frac{(1+\sqrt{b})-1+(1+\sqrt{b})E}{1-(1+\sqrt{b})E}$$

Hence, $$\sqrt{b+e} = \frac{\frac{\sqrt{b}}{1+\sqrt{b}}+E}{\frac{1}{1+\sqrt{b}}-E}, \tag{3}$$

For simplicity in symbols we may now let $\frac{1}{1+\sqrt{b}} = k$

Equation (3) then becomes $$\sqrt{b+e} = \frac{k\sqrt{b}+E}{k-E} \tag{4}$$

Now, to eliminate E in the denominator, both numerator and denominator of the right side of equation (4) are multiplied by $k^3+k^2E+kE^2+E^3$. The equation resulting is:

$$\sqrt{b+e}=\frac{k^4\sqrt{b}+k^2E+kE^2+E^3+E^4}{k^4-E^4}. \tag{5}$$

Squaring both sides of Equation (5)

$$b+e=\left(\frac{1}{k^8-2k^4E^4+E^8}\right)(k^8b+k^4E^2+k^2E^4+E^6+E^8+2k^6\sqrt{b}E$$
$$+2k^5\sqrt{b}E^2+2k^4\sqrt{b}E^3+2k^4\sqrt{b}E^4+2k^3E^3+2k^2E^4+2k^2E^5$$
$$+2kE^5+2kE^6+2E^7). \tag{6}$$

However, the value of E cannot exceed .5 and in case the sample from which b is computed includes 100 or more cases, it is highly improbable that E will exceed .03. Therefore, powers of E higher than E^3 may be disregarded since their values approximate zero. Equation (6) then becomes:

$$b+e=\frac{1}{k^8}(k^8b+2k^6\sqrt{b}E+k^4E^2+2k^5\sqrt{b}E^2+2k^4\sqrt{b}E^3+2k^3E^3)$$

Hence, $$e=\frac{2\sqrt{b}}{k^2}E+\left(\frac{1}{k^4}+\frac{2\sqrt{b}}{k^3}\right)E^2+\left(\frac{2\sqrt{b}}{k^4}+\frac{2}{k^5}\right)E^3. \tag{7}$$

Squaring, $$e^2=\frac{4b}{k^4}E^2+\left(\frac{1}{k^4}+\frac{2\sqrt{b}}{k^3}\right)^2E^4+\left(\frac{2\sqrt{b}}{k^4}+\frac{2}{k^5}\right)^2E^6$$
$$+\frac{4\sqrt{b}}{k^2}\left(\frac{1}{k^4}+\frac{2\sqrt{b}}{k^3}\right)E^3+\frac{4\sqrt{b}}{k^2}\left(\frac{2\sqrt{b}}{k^4}+\frac{2}{k^5}\right)E^4$$
$$+2\left(\frac{1}{k^4}+\frac{2\sqrt{b}}{k^3}\right)\left(\frac{2\sqrt{b}}{k^4}+\frac{2}{k^5}\right)E^5.$$

Summing this expression and disregarding values of $\sum E^3$, $\sum E^4$, $\sum E^5$, $\sum E^6$, all of which approximate zero, we have,

$$\sum e^2=\frac{4b}{k^4}\sum E^2.$$

But, since e is the error in estimating the proportion of b in the population, the standard deviation of $e=\sqrt{\frac{ab}{N}}$.

Then $$\sum e^2=N\left(\sqrt{\frac{ab}{N}}\right)^2=ab.$$

Hence, $$\sum E^2 = \frac{k^4ab}{4b} = \frac{k^4a}{4} = \frac{a}{4(1+\sqrt{b})^4} = \frac{1-\sqrt{b}}{4(1+\sqrt{b})^3}. \quad (8)$$

Now, to evaluate $\sum E$, let us go back to equation (7).

Summing this expression and disregarding values of $\sum E^3$, which approximate zero, we have:

$$\sum e = \frac{2\sqrt{b}}{k^2}\sum E + \left(\frac{1}{k^4} + \frac{2\sqrt{b}}{k^3}\right)\sum E^2.$$

But the sum of the errors in estimating b may be assumed to be zero, hence

$$\frac{2\sqrt{b}}{k^2}\sum E = -\left(\frac{1}{k^4} + \frac{2\sqrt{b}}{k^3}\right)\sum E^2.$$

Therefore $$\sum E = -\left(\frac{1}{2k^2\sqrt{b}} + \frac{1}{k}\right)\sum E^2.$$

Since $$\sum E^2 = \frac{k^4a}{4},$$

then $$\sum E = -\left(\frac{1}{2k^2\sqrt{b}} + \frac{1}{k}\right)\frac{k^4a}{4} = -\frac{k^2a}{8\sqrt{b}} - \frac{k^3a}{4}. \quad (9)$$

The standard deviation of E, $\sigma E = \sqrt{\frac{\Sigma E^2}{N} - \left(\frac{\Sigma E}{N}\right)^2}$.

Hence, $$\sigma E = \sqrt{\frac{k^4a}{4N} - \left(-\frac{k^2a}{8\sqrt{b}N} - \frac{k^3a}{4N}\right)^2}$$

$$= \sqrt{\frac{16k^4abN - k^4a^2 - 4k^5a^2\sqrt{b} - 4k^6a^2b}{64bN^2}}.$$

Since $k = \frac{1}{1+\sqrt{b}}$, and $a = (1-\sqrt{b})(1+\sqrt{b})$, then

$$\sigma E = \frac{1}{8N(1+\sqrt{b})}$$

$$\sqrt{\frac{16N(1-\sqrt{b})}{1+\sqrt{b}} - \frac{(1-\sqrt{b})^2}{b} - \frac{4(1-\sqrt{b})^2}{(1+\sqrt{b})^2\sqrt{b}} - \frac{4(1-\sqrt{b})^2}{(1+\sqrt{b})^2}}.$$

Then the P.E. of $\frac{q}{p+2q} = \frac{.6745}{8N(1+\sqrt{b})}$

$$\sqrt{\frac{16N(1-\sqrt{b})}{1+\sqrt{b}} - \frac{(1-\sqrt{b})^2}{b} - \frac{4(1-\sqrt{b})^2}{(1+\sqrt{b})^2\sqrt{b}} - \frac{4(1-\sqrt{b})^2}{(1+\sqrt{b})^2}}. \quad (10)$$

Since the sum of the last three terms under the radical is very small in

relation to the first term when N is 100 or more the last three terms may be omitted for approximate calculations.

In this case, P.E. of $\frac{q}{p+2q} = \frac{.6745(1-\sqrt{b})}{2(1+\sqrt{b})}\sqrt{\frac{1}{(1-b)N}}$.

DERIVATION OF A FORMULA FOR THE PROBABLE ERROR OF $\left(\frac{q}{p+2q}\right)^2$

In the previous section a formula for the probable error of $\frac{q}{p+2q}$ was developed. Using the same symbols as before the calculated value of $\left(\frac{q}{p+2q}\right)^2$ may be in error by an amount equal to: Calculated $\left(\frac{q}{p+2q}\right)^2$ $-$ True $\left(\frac{q}{p+2q}\right)^2$.

Hence, G, the error in $\left(\frac{q}{p+2q}\right)^2 = \left(\frac{\sqrt{b+e}}{1+\sqrt{b+e}}\right)^2 - \left(\frac{\sqrt{b}}{1+\sqrt{b}}\right)^2$. (11)

But since $E = \frac{\sqrt{b+e}}{1+\sqrt{b+e}} - \frac{\sqrt{b}}{1+\sqrt{b}}$, (Equation (1))

then $G = E^2 + \frac{2\sqrt{b}}{1+\sqrt{b}} E$. (12)

Summing, $\sum G = \sum E^2 + \frac{2\sqrt{b}}{1+\sqrt{b}} \sum E$.

Since $\sum E^2 = \frac{k^4 a}{4}$ (Equation (8))

and $\sum E = -\frac{k^2 a}{8\sqrt{b}} - \frac{k^3 a}{4}$ (Equation (9))

and $k = \frac{1}{1+\sqrt{b}}$,

then $\sum G = \frac{k^4 a}{4}(1-2\sqrt{b}) - \frac{k^3 a}{4}$. (13)

Squaring Equation (12): $G^2 = E^4 + \frac{4\sqrt{b}}{1+\sqrt{b}} E^3 + \frac{4b}{(1+\sqrt{b})^2} E^2$. (14)

Summing, $\sum G^2 = \sum E^4 + \frac{4\sqrt{b}}{1+\sqrt{b}} \sum E^3 + \frac{4b}{(1+\sqrt{b})^2} \sum E^2$.

However, we may disregard values of $\sum E^3$ and $\sum E^4$ since they approximate zero.

Hence, $$\sum G^2 = \frac{4b}{(1+\sqrt{b})^2} \sum E^2 = k^6 ab. \tag{15}$$

The standard deviation of G, $\sigma_G = \sqrt{\frac{\sum G^2}{N} - \left(\frac{\sum G}{N}\right)^2}$.

Therefore, $$\sigma_G = \sqrt{\frac{k^6 ab}{N} - \left(\frac{\frac{k^4 a}{4}(1-2\sqrt{b}) - \frac{k^3 a}{4}}{N}\right)^2}$$

$$\sigma_G = \frac{1-\sqrt{b}}{4N(1+\sqrt{b})^2} \sqrt{\frac{16Nb}{1-b} - 1 - \frac{(1-2\sqrt{b})^2}{(1+\sqrt{b})^2} + \frac{2(1-2\sqrt{b})}{1+\sqrt{b}}}. \tag{16}$$

Then, the P.E. of $\left(\frac{q}{p+2q}\right)^2$

$$= \frac{.6745(1-\sqrt{b})}{4N(1+\sqrt{b})^2} \sqrt{\frac{16Nb}{1-b} - 1 - \frac{(1-2\sqrt{b})^2}{(1+\sqrt{b})^2} + \frac{2(1-2\sqrt{b})}{1+\sqrt{b}}}. \tag{17}$$

Since the sum of the last three terms under the radical is very small in relation to the first term when N is 100 or more the last three terms may be omitted for approximate calculations.

In this case, P.E. of $\left(\frac{q}{p+2q}\right)^2 = \frac{.6745(1-\sqrt{b})}{(1+\sqrt{b})^2} \sqrt{\frac{b}{N(1-b)}}$.

I wish to thank Dr. RALPH TYLER for his kind assistance and suggestions in deriving these probable error formulae.

The table follows. It will be seen that values are given for proportions of recessives ranging from .001 to .999. For extremely rare dominants or recessives, where the proportion of recessives in the population is difficult to determine accurately, other methods of analysis must be used. These will be discussed in a later publication. In the table below,

B = proportion of recessive individuals in the general population,

R = proportion of recessive offspring to be expected in random matings of dominants with dominants, and

S = proportion of recessive offspring to be expected in random matings of dominants with recessives.

B	R	S	B	R	S	B	R	S	B	R	S
.001	.0009	.0307	.031	.0224	.1497	.061	.0392	.1981	.091	.0537	.2318
.002	.0018	.0428	.032	.0230	.1517	.062	.0398	.1994	.092	.0542	.2327
.003	.0027	.0519	.033	.0236	.1537	.063	.0402	.2006	.093	.0546	.2337
.004	.0035	.0595	.034	.0242	.1557	.064	.0408	.2019	.094	.0551	.2347
.005	.0044	.0660	.035	.0248	.1576	.065	.0413	.2032	.095	.0555	.2356
.006	.0052	.0719	.036	.0254	.1595	.066	.0418	.2044	.096	.0560	.2366
.007	.0060	.0772	.037	.0260	.1613	.067	.0423	.2056	.097	.0564	.2375
.008	.0067	.0821	.038	.0266	.1631	.068	.0428	.2068	.098	.0568	.2384
.009	.0075	.0867	.039	.0272	.1649	.069	.0433	.2080	.099	.0573	.2393
.010	.0083	.0909	.040	.0278	.1667	.070	.0438	.2092	.100	.0577	.2403
.011	.0090	.0949	.041	.0284	.1684	.071	.0443	.2104	.101	.0582	.2412
.012	.0098	.0987	.042	.0289	.1701	.072	.0448	.2116	.102	.0586	.2421
.013	.0105	.1024	.043	.0295	.1718	.073	.0452	.2127	.103	.0591	.2430
.014	.0112	.1058	.044	.0301	.1734	.074	.0457	.2139	.104	.0595	.2439
.015	.0119	.1091	.045	.0306	.1750	.075	.0462	.2150	.105	.0599	.2447
.016	.0126	.1123	.046	.0312	.1766	.076	.0467	.2161	.106	.0603	.2456
.017	.0133	.1153	.047	.0318	.1782	.077	.0472	.2172	.107	.0608	.2465
.018	.0140	.1183	.048	.0323	.1797	.078	.0477	.2183	.108	.0612	.2474
.019	.0147	.1211	.049	.0328	.1812	.079	.0481	.2194	.109	.0616	.2482
.020	.0154	.1239	.050	.0334	.1827	.080	.0486	.2205	.110	.0621	.2491
.021	.0160	.1266	.051	.0339	.1842	.081	.0491	.2216	.111	.0625	.2499
.022	.0167	.1292	.052	.0345	.1857	.082	.0496	.2226	.112	.0629	.2508
.023	.0174	.1317	.053	.0350	.1871	.083	.0500	.2237	.113	.0633	.2516
.024	.0180	.1341	.054	.0356	.1886	.084	.0505	.2247	.114	.0637	.2524
.025	.0186	.1365	.055	.0361	.1900	.085	.0509	.2257	.115	.0641	.2532
.026	.0193	.1389	.056	.0366	.1914	.086	.0514	.2268	.116	.0646	.2541
.027	.0199	.1411	.057	.0371	.1927	.087	.0519	.2278	.117	.0650	.2549
.028	.0205	.1433	.058	.0377	.1941	.088	.0524	.2288	.118	.0654	.2557
.029	.0212	.1455	.059	.0382	.1954	.089	.0529	.2298	.119	.0658	.2565
.030	.0218	.1476	.060	.0387	.1968	.090	.0533	.2308	.120	.0662	.2573

B	R	S	B	R	S	B	R	S	B	R	S
.121	.0666	.2581	.151	.0783	.2799	.181	.0891	.2985	.211	.0991	.3148
.122	.0670	.2589	.152	.0787	.2805	.182	.0894	.2990	.212	.0994	.3153
.123	.0674	.2597	.153	.0791	.2812	.183	.0898	.2996	.213	.0997	.3158
.124	.0678	.2604	.154	.0794	.2818	.184	.0901	.3002	.214	.1000	.3163
.125	.0682	.2612	.155	.0798	.2825	.185	.0905	.3008	.215	.1004	.3168
.126	.0686	.2620	.156	.0802	.2831	.186	.0908	.3013	.216	.1007	.3173
.127	.0690	.2627	.157	.0805	.2838	.187	.0911	.3019	.217	.1010	.3178
.128	.0694	.2635	.158	.0809	.2844	.188	.0915	.3025	.218	.1013	.3183
.129	.0699	.2643	.159	.0813	.2851	.189	.0918	.3030	.219	.1016	.3188
.130	.0702	.2650	.160	.0816	.2857	.190	.0922	.3036	.220	.1020	.3193
.131	.0707	.2658	.161	.0820	.2864	.191	.0925	.3041	.221	.1023	.3198
.132	.0710	.2665	.162	.0824	.2870	.192	.0928	.3047	.222	.1026	.3203
.133	.0714	.2672	.163	.0827	.2876	.193	.0932	.3052	.223	.1029	.3208
.134	.0718	.2680	.164	.0831	.2882	.194	.0935	.3058	.224	.1032	.3213
.135	.0722	.2687	.165	.0835	.2889	.195	.0938	.3063	.225	.1035	.3217
.136	.0726	.2694	.166	.0838	.2895	.196	.0942	.3069	.226	.1038	.3222
.137	.0730	.2702	.167	.0842	.2901	.197	.0945	.3074	.227	.1041	.3227
.138	.0734	.2709	.168	.0845	.2907	.198	.0949	.3079	.228	.1045	.3232
.139	.0738	.2716	.169	.0849	.2913	.199	.0952	.3085	.229	.1048	.3237
.140	.0742	.2723	.170	.0852	.2919	.200	.0955	.3090	.230	.1050	.3241
.141	.0745	.2730	.171	.0856	.2926	.201	.0959	.3096	.231	.1054	.3246
.142	.0749	.2737	.172	.0860	.2932	.202	.0962	.3101	.232	.1057	.3251
.143	.0753	.2744	.173	.0863	.2938	.203	.0965	.3106	.233	.1060	.3256
.144	.0756	.2751	.174	.0867	.2944	.204	.0968	.3111	.234	.1063	.3260
.145	.0761	.2758	.175	.0870	.2950	.205	.0972	.3117	.235	.1066	.3265
.146	.0765	.2765	.176	.0873	.2955	.206	.0975	.3122	.236	.1069	.3270
.147	.0768	.2772	.177	.0877	.2961	.207	.0978	.3127	.237	.1072	.3274
.148	.0772	.2778	.178	.0880	.2967	.208	.0981	.3132	.238	.1075	.3279
.149	.0776	.2785	.179	.0884	.2973	.209	.0984	.3137	.239	.1079	.3284
.150	.0780	.2792	.180	.0887	.2979	.210	.0988	.3143	.240	.1081	.3288

B	R	S	B	R	S	B	R	S	B	R	S
.241	.1084	.3293	.271	.1172	.3424	.301	.1255	.3543	.331	.1334	.3652
.242	.1087	.3297	.272	.1175	.3428	.302	.1257	.3547	.332	.1337	.3656
.243	.1090	.3302	.273	.1178	.3432	.303	.1260	.3550	.333	.1339	.3659
.244	.1093	.3306	.274	.1181	.3436	.304	.1263	.3554	.334	.1342	.3663
.245	.1096	.3311	.275	.1183	.3440	.305	.1266	.3558	.335	.1344	.3666
.246	.1099	.3315	.276	.1186	.3444	.306	.1268	.3562	.336	.1347	.3670
.247	.1102	.3320	.277	.1189	.3448	.307	.1271	.3565	.337	.1349	.3673
.248	.1105	.3324	.278	.1192	.3452	.308	.1274	.3569	.338	.1351	.3676
.249	.1108	.3329	.279	.1194	.3456	.309	.1277	.3573	.339	.1354	.3680
.250	.1111	.3333	.280	.1197	.3460	.310	.1280	.3577	.340	.1356	.3683
.251	.1114	.3338	.281	.1200	.3464	.311	.1282	.3580	.341	.1359	.3687
.252	.1117	.3342	.282	.1203	.3469	.312	.1285	.3584	.342	.1362	.3690
.253	.1120	.3347	.283	.1206	.3473	.313	.1287	.3588	.343	.1365	.3694
.254	.1123	.3351	.284	.1209	.3477	.314	.1290	.3591	.344	.1367	.3697
.255	.1126	.3355	.285	.1212	.3481	.315	.1292	.3595	.345	.1369	.3700
.256	.1129	.3360	.286	.1215	.3485	.316	.1295	.3599	.346	.1372	.3704
.257	.1132	.3364	.287	.1217	.3488	.317	.1297	.3602	.347	.1374	.3707
.258	.1134	.3368	.288	.1219	.3492	.318	.1300	.3606	.348	.1376	.3710
.259	.1138	.3373	.289	.1222	.3496	.319	.1303	.3609	.349	.1379	.3714
.260	.1140	.3377	.290	.1225	.3500	.320	.1305	.3613	.350	.1382	.3717
.261	.1143	.3381	.291	.1228	.3504	.321	.1308	.3617	.351	.1384	.3720
.262	.1147	.3386	.292	.1231	.3508	.322	.1311	.3620	.352	.1387	.3724
.263	.1149	.3390	.293	.1233	.3512	.323	.1313	.3624	.353	.1389	.3727
.264	.1152	.3394	.294	.1236	.3516	.324	.1316	.3627	.354	.1391	.3730
.265	.1155	.3398	.295	.1239	.3520	.325	.1318	.3631	.355	.1394	.3734
.266	.1158	.3403	.296	.1242	.3524	.326	.1321	.3635	.356	.1397	.3737
.267	.1161	.3407	.297	.1244	.3528	.327	.1324	.3638	.357	.1399	.3740
.268	.1164	.3411	.298	.1247	.3531	.328	.1326	.3642	.358	.1402	.3744
.269	.1166	.3415	.299	.1250	.3535	.329	.1329	.3645	.359	.1404	.3747
.270	.1169	.3419	.300	.1253	.3539	.330	.1332	.3649	.360	.1406	.3750

B	R	S	B	R	S	B	R	S	B	R	S
.361	.1409	.3753	.391	.1480	.3847	.421	.1548	.3935	.451	.1614	.4018
.362	.1412	.3757	.392	.1482	.3850	.422	.1551	.3938	.452	.1616	.4020
.363	.1414	.3760	.393	.1485	.3853	.423	.1553	.3941	.453	.1619	.4023
.364	.1416	.3763	.394	.1487	.3856	.424	.1555	.3944	.454	.1621	.4026
.365	.1418	.3766	.395	.1489	.3859	.425	.1557	.3946	.455	.1623	.4028
.366	.1421	.3769	.396	.1492	.3862	.426	.1560	.3949	.456	.1625	.4031
.367	.1424	.3773	.397	.1494	.3865	.427	.1562	.3952	.457	.1627	.4034
.368	.1426	.3776	.398	.1496	.3868	.428	.1564	.3955	.458	.1629	.4036
.369	.1428	.3779	.399	.1499	.3871	.429	.1566	.3958	.459	.1631	.4039
.370	.1430	.3782	.400	.1501	.3874	.430	.1568	.3960	.460	.1633	.4041
.371	.1433	.3785	.401	.1503	.3877	.431	.1571	.3963	.461	.1635	.4044
.372	.1436	.3789	.402	.1505	.3880	.432	.1573	.3966	.462	.1637	.4047
.373	.1438	.3792	.403	.1508	.3883	.433	.1575	.3969	.463	.1639	.4049
.374	.1440	.3795	.404	.1510	.3886	.434	.1577	.3972	.464	.1642	.4052
.375	.1443	.3798	.405	.1512	.3889	.435	.1579	.3974	.465	.1644	.4054
.376	.1445	.3801	.406	.1515	.3892	.436	.1582	.3977	.466	.1646	.4057
.377	.1447	.3804	.407	.1517	.3895	.437	.1584	.3980	.467	.1648	.4060
.378	.1450	.3807	.408	.1519	.3898	.438	.1586	.3983	.468	.1650	.4062
.379	.1452	.3811	.409	.1522	.3901	.439	.1588	.3984	.469	.1652	.4065
.380	.1455	.3814	.410	.1524	.3904	.440	.1590	.3988	.470	.1654	.4067
.381	.1457	.3817	.411	.1526	.3907	.441	.1592	.3991	.471	.1656	.4070
.382	.1459	.3820	.412	.1528	.3909	.442	.1594	.3993	.472	.1658	.4072
.383	.1462	.3823	.413	.1530	.3912	.443	.1597	.3996	.473	.1661	.4075
.384	.1464	.3826	.414	.1533	.3915	.444	.1599	.3999	.474	.1663	.4078
.385	.1466	.3829	.415	.1535	.3918	.445	.1601	.4002	.475	.1665	.4080
.386	.1468	.3832	.416	.1537	.3921	.446	.1603	.4004	.476	.1667	.4083
.387	.1471	.3835	.417	.1540	.3924	.447	.1606	.4007	.477	.1669	.4085
.388	.1473	.3838	.418	.1542	.3927	.448	.1608	.4010	.478	.1671	.4088
.389	.1475	.3841	.419	.1544	.3930	.449	.1610	.4012	.479	.1673	.4090
.390	.1478	.3844	.420	.1546	.3932	.450	.1612	.4015	.480	.1675	.4093

B	R	S	B	R	S	B	R	S	B	R	S
.481	.1677	.4095	.511	.1738	.4169	.541	.1796	.4238	.571	.1852	.4304
.482	.1679	.4098	.512	.1740	.4171	.542	.1798	.4240	.572	.1854	.4306
.483	.1681	.4100	.513	.1742	.4173	.543	.1800	.4243	.573	.1856	.4308
.484	.1683	.4103	.514	.1744	.4176	.544	.1802	.4245	.574	.1858	.4311
.485	.1685	.4105	.515	.1746	.4178	.545	.1804	.4247	.575	.1860	.4313
.486	.1687	.4108	.516	.1748	.4180	.546	.1806	.4249	.576	.1862	.4315
.487	.1689	.4110	.517	.1750	.4183	.547	.1808	.4252	.577	.1864	.4317
.488	.1691	.4113	.518	.1752	.4185	.548	.1810	.4254	.578	.1865	.4319
.489	.1693	.4115	.519	.1754	.4188	.549	.1811	.4256	.579	.1867	.4321
.490	.1695	.4118	.520	.1756	.4190	.550	.1813	.4258	.580	.1869	.4323
.491	.1697	.4120	.521	.1757	.4192	.551	.1815	.4260	.581	.1871	.4325
.492	.1700	.4123	.522	.1760	.4195	.552	.1817	.4263	.582	.1873	.4328
.493	.1702	.4125	.523	.1762	.4197	.553	.1819	.4265	.583	.1875	.4330
.494	.1704	.4128	.524	.1763	.4199	.554	.1821	.4267	.584	.1877	.4332
.495	.1706	.4130	.525	.1765	.4202	.555	.1823	.4269	.585	.1878	.4334
.496	.1708	.4132	.526	.1767	.4204	.556	.1825	.4272	.586	.1880	.4336
.497	.1710	.4135	.527	.1769	.4206	.557	.1827	.4274	.587	.1882	.4338
.498	.1712	.4137	.528	.1771	.4208	.558	.1828	.4276	.588	.1884	.4340
.499	.1714	.4140	.529	.1773	.4211	.559	.1830	.4278	.589	.1885	.4342
.500	.1716	.4142	.530	.1775	.4213	.560	.1832	.4280	.590	.1887	.4344
.501	.1718	.4145	.531	.1777	.4215	.561	.1834	.4282	.591	.1889	.4346
.502	.1720	.4147	.532	.1779	.4218	.562	.1836	.4285	.592	.1891	.4348
.503	.1722	.4149	.533	.1781	.4220	.563	.1838	.4287	.593	.1893	.4351
.504	.1724	.4152	.534	.1783	.4222	.564	.1840	.4289	.594	.1895	.4353
.505	.1726	.4154	.535	.1785	.4225	.565	.1841	.4291	.595	.1897	.4355
.506	.1728	.4157	.536	.1787	.4227	.566	.1843	.4293	.596	.1898	.4357
.507	.1730	.4159	.537	.1788	.4229	.567	.1845	.4296	.597	.1900	.4359
.508	.1732	.4161	.538	.1790	.4231	.568	.1847	.4298	.598	.1902	.4361
.509	.1734	.4164	.539	.1792	.4234	.569	.1849	.4300	.599	.1904	.4363
.510	.1736	.4166	.540	.1794	.4236	.570	.1851	.4302	.600	.1905	.4365

B	R	S	B	R	S	B	R	S	B	R	S
.601	.1907	.4367	.631	.1960	.4427	.661	.2011	.4484	.691	.2060	.4539
.602	.1909	.4369	.632	.1962	.4429	.662	.2012	.4486	.692	.2062	.4541
.603	.1911	.4371	.633	.1963	.4431	.663	.2014	.4488	.693	.2064	.4543
.604	.1912	.4373	.634	.1965	.4433	.664	.2016	.4490	.694	.2066	.4545
.605	.1914	.4375	.635	.1967	.4435	.665	.2019	.4493	.695	.2067	.4547
.606	.1916	.4377	.636	.1969	.4437	.666	.2020	.4494	.696	.2068	.4548
.607	.1918	.4379	.637	.1971	.4439	.667	.2021	.4496	.697	.2070	.4550
.608	.1919	.4381	.638	.1972	.4441	.668	.2022	.4497	.698	.2072	.4552
.609	.1921	.4383	.639	.1974	.4443	.669	.2024	.4499	.699	.2074	.4554
.610	.1923	.4385	.640	.1975	.4444	.670	.2026	.4501	700	.2075	.4555
.611	.1925	.4387	.641	.1977	.4446	.671	.2028	.4503	.701	.2077	.4557
.612	.1926	.4389	.642	.1978	.4448	.672	.2030	.4505	.702	.2078	.4559
.613	.1928	.4391	.643	.1980	.4450	.673	.2031	.4507	.703	.2080	.4561
.614	.1930	.4393	.644	.1982	.4452	.674	.2033	.4509	.704	.2081	.4562
.615	.1932	.4395	.645	.1984	.4454	.675	.2034	.4510	.705	.2083	.4564
.616	.1933	.4397	.646	.1986	.4456	.676	.2036	.4512	.706	.2085	.4566
.617	.1935	.4399	.647	.1987	.4458	.677	.2038	.4514	.707	.2087	.4568
.618	.1937	.4401	.648	.1989	.4460	.678	.2039	.4516	.708	.2088	.4569
.619	.1939	.4403	.649	.1991	.4462	.679	.2041	.4518	.709	.2089	.4571
.620	.1940	.4405	.650	.1993	.4464	.680	.2042	.4519	.710	.2091	.4573
.621	.1942	.4407	.651	.1994	.4466	.681	.2044	.4521	.711	.2093	.4575
.622	.1944	.4409	.652	.1995	.4467	.682	.2046	.4523	.712	.2094	.4576
.623	.1946	.4411	.653	.1997	.4469	.683	.2048	.4525	.713	.2096	.4578
.624	.1948	.4413	.654	.1999	.4471	.684	.2049	.4527	.714	.2098	.4580
.625	.1949	.4415	.655	.2001	.4473	.685	.2051	.4529	.715	.2100	.4582
.626	.1951	.4417	.656	.2003	.4475	.686	.2052	.4530	.716	.2100	.4583
.627	.1953	.4419	.657	.2004	.4477	.687	.2054	.4532	.717	.2102	.4585
.628	.1955	.4421	.658	.2006	.4479	.688	.2056	.4534	.718	.2104	.4587
.629	.1956	.4423	.659	.2008	.4881	.689	.2058	.4536	.719	.2106	.4589
.630	.1958	.4425	.660	.2010	.4483	.690	.2059	.4538	.720	.2107	.4590

B	R	S	B	R	S	B	R	S	B	R	S
.721	.2109	.4592	.751	.2156	.4643	.781	.2201	.4691	.811	.2245	.4738
.722	.2111	.4594	.752	.2157	.4644	.782	.2202	.4693	.812	.2247	.4740
.723	.2112	.4595	.753	.2158	.4646	.783	.2204	.4695	.813	.2249	.4742
.724	.2113	.4597	.754	.2160	.4648	.784	.2205	.4696	.814	.2250	.4743
.725	.2115	.4599	.755	.2161	.4649	.785	.2207	.4698	.815	.2252	.4745
.726	.2117	.4601	.756	.2163	.4651	.786	.2208	.4699	.816	.2253	.4746
.727	.2118	.4602	.757	.2165	.4653	.787	.2210	.4701	.817	.2254	.4748
.728	.2120	.4604	.758	.2166	.4654	.788	.2212	.4703	.818	.2255	.4749
.729	.2122	.4606	.759	.2168	.4656	.789	.2213	.4704	.819	.2257	.4751
.730	.2123	.4607	.760	.2170	.4658	.790	.2215	.4706	.820	.2258	.4752
.731	.2124	.4609	.761	.2171	.4659	.791	.2216	.4707	.821	.2260	.4754
.732	.2126	.4611	.762	.2173	.4661	.792	.2218	.4709	.822	.2261	.4755
.733	.2128	.4613	.763	.2174	.4662	.793	.2219	.4710	.823	.2263	.4757
.734	.2129	.4614	.764	.2175	.4664	.794	.2220	.4712	.824	.2264	.4758
.735	.2131	.4616	.765	.2177	.4666	.795	.2222	.4714	.825	.2266	.4760
.736	.2133	.4618	.766	.2178	.4667	.796	.2223	.4715	.826	.2267	.4761
.737	.2134	.4619	.767	.2180	.4669	.797	.2225	.4716	.827	.2269	.4763
.738	.2135	.4621	.768	.2182	.4671	.798	.2226	.4718	.828	.2270	.4764
.739	.2137	.4623	.769	.2183	.4672	.799	.2228	.4719	.829	.2272	.4766
.740	.2138	.4624	.770	.2185	.4674	.800	.2229	.4721	.830	.2273	.4767
.741	.2140	.4626	.771	.2186	.4675	.801	.2231	.4723	.831	.2274	.4769
.742	.2142	.4628	.772	.2187	.4677	.802	.2233	.4725	.832	.2275	.4770
.743	.2143	.4629	.773	.2189	.4679	.803	.2234	.4726	.833	.2277	.4772
.744	.2145	.4631	.774	.2190	.4680	.804	.2235	.4728	.834	.2278	.4773
.745	.2147	.4633	.775	.2192	.4682	.805	.2236	.4729	.835	.2280	.4775
.746	.2148	.4634	.776	.2193	.4683	.806	.2238	.4731	.836	.2281	.4776
.747	.2149	.4636	.777	.2195	.4685	.807	.2239	.4732	.837	.2283	.4778
.748	.2151	.4638	.778	.2197	.4687	.808	.2241	.4734	.838	.2284	.4779
.749	.2152	.4639	.779	.2198	.4688	.809	.2242	.4735	.839	.2286	.4781
.750	.2154	.4641	.780	.2200	.4690	.810	.2244	.4737	.840	.2287	.4782

B	R	S	B	R	S	B	R	S	B	R	S
.841	.2289	.4784	.871	.2330	.4827	.901	.2372	.4870	.931	.2412	.4910
.842	.2290	.4785	.872	.2332	.4829	.902	.2373	.4871	.932	.2413	.4912
.843	.2292	.4787	.873	.2333	.4830	.903	.2375	.4873	.933	.2414	.4913
.844	.2293	.4788	.874	.2335	.4832	.904	.2376	.4874	.934	.2416	.4915
.845	.2294	.4790	.875	.2336	.4833	.905	.2377	.4875	.935	.2417	.4916
.846	.2295	.4791	.876	.2338	.4835	.906	.2379	.4877	.936	.2418	.4917
.847	.2297	.4793	.877	.2339	.4836	.907	.2380	.4878	.937	.2420	.4919
.848	.2298	.4794	.878	.2340	.4837	.908	.2381	.4879	.938	.2421	.4920
.849	.2300	.4796	.879	.2342	.4839	.909	.2382	.4881	.939	.2422	.4921
.850	.2301	.4797	.880	.2343	.4840	.910	.2383	.4882	.940	.2424	.4923
.851	.2302	.4798	.881	.2345	.4842	.911	.2385	.4884	.941	.2425	.4924
.852	.2304	.4800	.882	.2346	.4843	.912	.2386	.4885	.942	.2426	.4925
.853	.2305	.4801	.883	.2347	.4845	.913	.2387	.4886	.943	.2428	.4926
.854	.2307	.4803	.884	.2348	.4846	.914	.2389	.4888	.944	.2429	.4928
.855	.2309	.4804	.885	.2349	.4847	.915	.2390	.4889	.945	.2430	.4929
.856	.2310	.4806	.886	.2351	.4849	.916	.2391	.4890	.946	.2432	.4931
.857	.2311	.4807	.887	.2352	.4850	.917	.2393	.4892	.947	.2433	.4932
.858	.2313	.4809	.888	.2354	.4852	.918	.2394	.4893	.948	.2434	.4933
.859	.2314	.4810	.889	.2355	.4853	.919	.2395	.4894	.949	.2435	.4935
.860	.2316	.4812	.890	.2356	.4854	.920	.2397	.4896	.950	.2436	.4936
.861	.2317	.4813	.891	.2358	.4856	.921	.2398	.4897	.951	.2437	.4937
.862	.2318	.4815	.892	.2359	.4857	.922	.2400	.4899	.952	.2439	.4939
.863	.2319	.4816	.893	.2361	.4859	.923	.2401	.4900	.953	.2440	.4940
.864	.2320	.4817	.894	.2362	.4860	.924	.2402	.4901	.954	.2441	.4941
.865	.2322	.4819	.895	.2363	.4861	.925	.2404	.4903	.955	.2442	.4942
.866	.2323	.4820	.896	.2365	.4863	.926	.2405	.4904	.956	.2444	.4944
.867	.2325	.4822	.897	.2366	.4864	.927	.2406	.4905	.957	.2445	.4945
.868	.2326	.4823	.898	.2368	.4866	.928	.2408	.4907	.958	.2446	.4946
.869	.2328	.4825	.899	.2369	.4867	.929	.2409	.4908	.959	.2448	.4948
.870	.2329	.4826	.900	.2370	.4868	930	.2410	.4909	.960	.2449	.4949

B	R	S	B	R	S	B	R	S	B	R	S
.961	.2450	.4950	.971	.2463	.4963	.981	.2476	.4976	.991	.2489	.4989
.962	.2452	.4952	.972	.2465	.4965	.982	.2477	.4977	.992	.2490	.4990
.963	.2453	.4953	.973	.2466	.4966	.983	.2479	.4979	.993	.2491	.4991
.964	.2454	.4954	.974	.2467	.4967	.984	.2480	.4980	.994	.2493	.4993
.965	.2456	.4956	.975	.2468	.4968	.985	.2481	.4981	.995	.2494	.4994
.966	.2457	.4957	.976	.2470	.4970	.986	.2482	.4982	.996	.2495	.4995
.967	.2458	.4958	.977	.2471	.4971	.987	.2484	.4984	.997	.2496	.4996
.968	.2459	.4959	.978	.2472	.4972	.988	.2485	.4985	.998	.2498	.4998
.969	.2461	.4961	.979	.2474	.4974	.989	.2486	.4986	.999	.2499	.4999
.970	.2462	.4962	.980	.2475	.4975	.990	.2487	.4987			

THE EFFECT OF A SUPERNUMERARY X CHROMOSOME ON MEMBERS OF THE BAR SERIES OF DROSOPHILA[1]

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Studies on the bar series of Drosophila have, until the present time, been carried out entirely on flies of normal diploid chromosome constitution. The phenomena of polyploidy and heteroploidy make possible an investigation of the expression of the bar gene and its allelomorphs in flies having an aberrant chromosome complex.

As a result of occasional failure of the X chromosomes to disjoin during maturation of the egg, individuals having three X chromosomes and the normal complement of autosomes may be produced. These individuals, known as superfemales, are of extremely low viability, and die at various stages of development, rarely attaining maturity (LI 1927).

L. V. MORGAN (1922) isolated a yellow stock in which the X chromosomes of the female appeared to be permanently attached. In addition to the attached X chromosomes, a Y from the father was present. It was found that occasionally the X's disjoin, giving normal diploid daughters in which one X comes from the mother, the other from the father.

When double-yellow attached-X females ($\widehat{yy}$) are mated to normal males, a small percentage of superfemales may be expected among the progeny. Furthermore, as the result of infrequent disjunction of the X's, normal diploid females are produced. Since these two classes of flies differ only in the presence or absence of an extra X chromosome, a comparison of the two affords an excellent medium for a study of the effect of a supernumerary X chromosome on the expression of any character.

EXPERIMENTAL PROCEDURE

Three members of the bar series were studied: (1) bar (*B*); (2) double-bar (*BB*); (3) infra-bar (B^i).

Bar series. The flies on which facet counts were made were obtained in an experiment of HERSH, KARRER, and LOOMIS (1930) on the possible effect of supersonic vibrations on mutation rate. In the experiment *ct f B* males were inbred with $\widehat{yy}$ females for eight generations. The males were

[1] This investigation was carried out at the Biological Laboratory of WESTERN RESERVE UNIVERSITY. The author wishes to acknowledge his indebtedness to DR. A. H. HERSH for suggesting the problem, as well as for his interest and unstinting co-operation throughout the course of the work. He also wishes to thank DR. C. R. PLUNKETT for many helpful suggestions in the interpretation of the data.

then subjected to treatment of 285,000 vibrations per second and mated to $\widehat{yy}$ females in pair matings.

Among the progeny of these flies were 290 superfemales, and 30 heterozygous females resulting from disjunction of the X chromosomes. Facet counts were made on these two classes, and also on a number of superfemales obtained from control matings in the experiment. The frequency distribution in the different progeny classes is given in table 1.

Double-bar series. f *BB* males were inbred with $\widehat{yy}$ females for six generations. As in the bar series, pair matings were used throughout the experiment (see table 1).

Infra-bar series. f B^i males were inbred with $\widehat{yy}$ females for four generations. Pair matings were used in the experiment (see table 1).

Temperature control. In all the experiments the flies were raised at 25°C. In the bar series, several times in the course of the experiment, the temperature rose to 28°C for short intervals, but never fell below 25°C. In the *BB* and B^i series the average fluctuation was ± 0.5°C. On a few occasions and for very short periods, the temperature varied as much as 2°C.

Culture medium. In all the experiments a 1 percent banana-agar medium in 8 dram vials was used. The food was inoculated with a small amount of yeast foam. The parent flies were permitted to lay eggs over a 6 to 7 day period, and then transferred to fresh vials. Two or three broods were obtained from each set of parents. The progeny were examined and counted every third day over a 10 to 15 day period after emergence of the first fly from the pupa case. The superfemales are easily recognized by a number of definite and constant characteristics (BRIDGES 1922). The disjunctional females, on the other hand, are quite normal in appearance, but may be identified by the gray body color and heterozygous eye.

Facet counts.[2] In the *BB* series, facet counts were made directly, using a binocular dissecting microscope with 10× eyepiece and 24 mm objective. In the *B* and B^i series dissections of the cornea were made, following a modification of the procedure of R. K. HERSH (1924) and STURTEVANT (1925). The flies were placed in a 5 percent solution of KOH for 24–48 hours for clearing. The cornea was then removed by fine needles and mounted in water upon a slide. Three or four radial incisions were made in order to flatten the somewhat convex surface. The cornea was then projected on a Bausch and Lomb projecting apparatus and the facets counted. Error was minimized by blocking out each facet as counted. The facet counts were checked by making recounts of 12 eyes in each series, a few days after the initial counts. In all cases the error was found to be slightly less than 1 percent.

[2] The facet counts in the bar series were made by Miss EDITH I. SCRIBNER, who has kindly consented to publication of the data in this paper.

Temperature variations have already been considered. They occurred quite at random, and did not appear to be of such magnitude as to obscure any marked effect on facet number.

No effort was made to control the number of eggs laid per vial. Considering the length of the egg-laying period a crowded condition no doubt prevailed. This condition, however, was constant in all the experiments. LUCE (1931) has shown that crowding markedly reduced facet number in B^i. Whether crowding might act differentially in diplo- and triplo-X flies is unknown.

EXPERIMENTAL DATA

In table 1 the progeny results are summarized. The last column of the table gives the data of DOBZHANSKY (1928) on the effect of temperature on production of superfemales. These data are for 24.5°C, a temperature comparable with the one used in the present experiment. In DOBZHANSKY'S experiments sooty males were mated to $\widehat{yy}$ females.

TABLE 1

Table showing frequency distribution of individuals in progeny.

	B SERIES	BB SERIES	B^i SERIES	DATA OF DOBZHANSKY AT 24.5° C
$\widehat{yy}$ females	12,189	19,054	4,625	3,262
f males	13,598	17,184	5,261	3,645
Superfemales	290	21	61	101
Yellow males	28	21	1	1
Disjunctional females	30	22	2*	2
Total	26,135	36,302	9,950	7,011
Percent superfemales	1.11	0.058	0.61	1.4
Percent disjunction	0.22	0.12	0.03	0.04

* These two females were inbred to fB^i brothers in order to obtain a sufficient number of diploid heterozygous females for facet counts.

It is apparent that large differences in relative numbers of superfemales, and also in the frequency of disjunction of X chromosomes, occur in the three series. These differences may be explained by the fact that the system of inbreeding used was designed primarily to render the flies of any one series isogenic with each other. It did not eliminate genetic differences between members of different series to any appreciable extent. It is likewise necessary to consider possible direct effects of the different bar allelomorphs on viability and disjunction. These allelomorphs are known to affect viability in different degree.

When the production rate of superfemales in the different series is compared with DOBZHANSKY'S data, there is, in general, considerable disparity.

His data, however, are in fairly close agreement with the results on the bar series. As regards disjunction rate, DOBZHANSKY's data accord well with the data in the infra-bar series. These comparisons, however, have little meaning due to the genetic heterogeneity of the flies in the two experiments.

In table 2 are summarized the essential statistics on facet number in the three series, together with their standard errors.

TABLE 2

Summary of data on facet number.

	B^i SUPER ♀	B^i HET. ♀	*BB* SUPER ♀	*BB* HET. ♀	*B* SUPER ♀	*B* HET. ♀
Mean facet number	558.5 ±12.1	475.0 ±11.7	70.8 ±2.7	44.4 ±1.1	550.8 ±4.3	395.3 ±18.8
Standard deviation	85.5 ±8.6	82.5 ±8.3	11.2 ±1.9	8.1 ±0.8	78.3 ±3.1	90.0 ±13.2
Coefficient of variability	15.3	17.4	15.8	18.4	14.2	22.8
Number of flies	50	50	17	53	327	23

In each of the three series, a supernumerary X chromosome produces a significant increase in facet number, taking the diplo-X heterozygous female as base of reference. The percentage increase, however, differs for the three bar allelomorphs. It is 16.7 percent for B^i, 39.4 percent for *B*, and 59.2 percent for *BB*. However, as the table shows, the order of absolute increase in facet number as affected by a supernumerary X chromosome is $B > B^i > BB$.

DISCUSSION

The simplest and most apparent interpretation of the increase in facet number produced by an additional X chromosome, would be that in the triplo-X fly there are present two wild-type allelomorphs of bar, as compared with one such allelomorph in the diploid heterozygous female. If we look upon the wild-type allelomorph of bar as a differential for facet production, the increase in facet number is to be expected. Evidence, however, has been adduced (BRIDGES 1917, STURTEVANT 1925) that the normal allelomorph of bar plays no part in facet production. STURTEVANT has suggested the possibility that no normal allelomorph of bar exists. In either of these cases the data require an alternative explanation. The wild-type eye is the resultant of the interaction of a number of genes distributed throughout the chromosomes. Some of these genes may well have their loci in the X chromosome (HERSH 1929). From this standpoint of

genic balance, the eye which is produced is the somatic expression of a balance among the effects of these genes, some having positive and others negative effects on facet production. If in the X chromosome the internal balance is such that the net tendency is in the plus direction facets are added. Conversely if minus modifiers preponderate, facet number is reduced. From the data here cited it appears that the balance of modifiers in the X chromosomes of the stocks investigated favor facet production.

How does this interpretation relate itself to other studies on facet number? All studies on members of the bar series have shown that males have somewhat larger eyes than the homozygous females. In considering the effect of the addition of an X chromosome it is necessary that we use the heterozygous and not the homozygous female as base of reference. The reason for this is at once apparent. The bar gene and its allelomorphs are powerful inhibitors of facet production. The presence of two bar genes in the female as against one in the male easily obscures whatever effect the additional X chromosome in the female might have on facet production. Females heterozygous for any allelomorph of the bar series always have much larger eyes than males having the same allelomorph. Here then the only differential is an additional X chromosome in the female, and the results are explained by a preponderance of plus modifiers in this chromosome.

The data available on the wild-type eye, however, are somewhat conflicting. R. K. HERSH (1924) in a study over a range of temperatures found that the wild-type eye is very slightly larger in the male than in the female at all temperatures studied. STURTEVANT (1925), on the other hand, observed that at 25°C the female eye is significantly larger than the male eye. The data of KRAFKA (1920) are extremely meager but interesting insofar as they may be used to corroborate the results of either of the above investigators. If KRAFKA's experiments are taken singly, his one experiment, in which a sufficient number of flies was used, is in accord with R. K. HERSH. However, when all data at one temperature are grouped they confirm STURTEVANT.

The discrepancies in the data on the wild-type eye are not readily attributable to error on the part of any of the investigators. In all experiments the flies were inbred for a number of generations. The technique in facet counting was such as to give a high order of precision to the counts. The differences no doubt arise from genetic differences in the stocks used. The disparity in the data is suggestive of the possibility of a reversal in sexual dimorphism. ZELENY (1921) has shown that there is a marked reduction in sexual dimorphism in bar resulting from selection. KRAFKA's data (1920) on *BB* indicate further that sexual dimorphism is a function of external factors. In the experiments there is a progressive decrease in

sexual dimorphism of the eye with increase in temperature, and a possible reversal between 29°C and 31°C.

The interpretation of the results presented in this paper, namely—that an additional X chromosome increases facet number because in the X chromosome the plus modifiers of facet number predominate— is consonant with all existing data on the bar series. The conflicting data on the wild-type eye, when compared with the condition in the bar series, indicate that we may be dealing with two totally or partially different systems of eye modifiers in the X chromosomes. The one system is stable as evidenced by the consistency of all data on the bar series which show that the eye of the heterozygous bar female is considerably larger than the male. This system may, as seems probable, consist of a single plus modifier acting directly on the bar system, and appears to be a constant feature of all stocks investigated. The second system is labile, due, presumably, to the fact that it consists of a number of minor modifiers mutating at random either in a plus or minus direction, and appears to act on the general facet-producing mechanism. This second system accounts for the disparity in the results of the investigators on facet number in the wild-type eye.

SUMMARY

1. The effect on facet number of a supernumerary wild-type X chromosome is reported for three members of the bar series, namely, bar, double-bar, and infra-bar. The facet number in superfemales, having two wild-type X chromosomes and one X chromosome carrying bar or one of its allelomorphs, is compared with the facet number in normal diploid flies heterozygous for the same allelomorph.

2. In each of the three series, a supernumerary wild-type X chromosome increases facet number markedly.

3. The results are interpreted in terms of genic balance. In the X chromosome plus modifiers of facet number predominate, thereby increasing facet production.

4. This conclusion is tested by comparing males showing one of the bar allelomorphs with females heterozygous for the same allelomorph, and in all cases the interpretation is tenable.

5. With respect to sexual dimorphism in the wild-type, the data are conflicting, indicating that we are dealing with different systems of facet number modifiers in the wild-type stocks and in bar and its allelomorphs.

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THE EFFECT OF CONJUGATION WITHIN A CLONE OF EUPLOTES PATELLA

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INTRODUCTION

The present paper deals with an investigation of the effects of conjugation in the hypotrichous ciliate *Euplotes patella*, and represents the first attempt to examine the genetics of this form. It is concerned with the fundamental question of the gross effects of conjugation within a clone. Variability in fission rate among ex-conjugants as compared with that among the parent non-conjugants is the central problem of the study, although other related questions of similar interest are examined and discussed.

JENNINGS (1913), RAFFEL (1930), and JENNINGS, RAFFEL, LYNCH and SONNEBORN (1932), have shown that when the genetically similar members of a clone of Paramecium undergo conjugation, the resulting exconjugants exhibit a wide range of diversities in their inherited characteristics. They conclude that conjugation breaks up a single clone into many new clones.

The desirability of examining this matter in other Protozoa has prompted the present investigation. It was suggested by Professor H. S. JENNINGS, for whose supervision and helpful criticism the writer wishes to record his appreciation. Thanks are also due to Dr. RUTH S. LYNCH for assistance in part of the experimental work, and for aid in the preparation of this paper.

THE ORGANISM

Euplotes patella, the organism used in this work, has proved to be an extremely favorable form for this type of investigation. Its comparatively large size makes possible the observation and handling of individuals without great difficulty, and permits the use of the lower powers of the dissecting binocular with its attendant advantages. Furthermore, it can be easily cultivated in the laboratory, and lives well in mass cultures and as isolation lines on slides. Conjugation can generally be induced readily, and as far as is known, endomixis takes place only during encystment. The greatest advantage that Euplotes affords, however, lies in the fact that it is carnivorous and flourishes on a diet of Chilomonas or other flagellates of similar size. Because of this fact it is comparatively simple to control qualitatively the food factor of the environment. With bacteria-eating protozoa such control offers very great difficulties, and at times has stood in the way of obtaining valid results.

CULTURE METHOD

The fact that Euplotes was found to live extremely well in the same fluid in which its food organism, Chilomonas, grew abundantly, made it possible to use a simple technique in the cultivation of the ciliate. The Chilomonas were grown in an infusion of rye grains made up fresh each day by boiling 2 grams of the rye in 200 cc of Chattolanee spring water for ten minutes in a 250 cc Pyrex beaker. This was filtered while still hot through Eaton and Dikeman filter paper into another 250 cc Pyrex beaker in which it was allowed to stand, covered, for 24 hours. At the end of this time it was placed in a finger bowl along with twelve of the boiled rye grains and was then inoculated with 30 cc of the six day old Chilomonas culture, which remained from the fluid used that day on the slides for the experimental lines. After six days the culture made up thus was swarming with clouds of vigorous, active Chilomonas. Where these clouds were thick, the culture was almost entirely free of bacterial debris, and from such regions the fluid rich in Chilomonas was pipetted into a 50 cc Pyrex beaker. From there it was used directly on the slides as the culture medium for the Euplotes.

The slides used were the ordinary two-depression culture slides, all uniformly made of white German glass and procured from the same source. The concavities were large enough to hold, without danger of overflowing, two drops of culture fluid, the quantity used consistently throughout the investigation. Before putting the drops on the slides, the fluid in the beaker was thoroughly mixed by gentle pipetting, and in making the drops the pipette was always held vertically. These precautions were taken to insure every line's getting approximately the same quantity of Chilomonas. This quantity was always a large excess over that needed to feed the Euplotes during the twenty-four hours between transfers.

The Euplotes were transferred by means of a capillary pipette. Mass cultures were kept in small flat Columbia dishes which hold about one cc of fluid. These and the experimental lines on slides were kept in the usual moist chambers on glass plates supported above distilled water by glass supports. All the moist chambers used in the experiment were subjected to the same temperature variation; that is, any temperature changes that occurred during the experiment were experienced by all the moist chambers at the same time. The total range of these changes was from 19.3° to 23.8°.

THE EXPERIMENT

In *Euplotes patella*, conjugation brings about temporary but fundamental and striking changes in the organism's morphology and behavior. Observation of several hundreds of conjugants has shown that in most cases the members of conjugating pairs have separated on the day follow-

ing conjugation, and after separating appear smaller, weaker, and less active than non-conjugants. The significance of these changes was first discussed, on the basis of his observations on *Euplotes charon*, by ENGELMANN (1862), and later in great detail, with regard to *Euplotes patella*, by MAUPAS (1889). From their accounts and figures it appears that there occurs at this time a loss of many differentiated parts such as cirri, oral membranelles, and mouth. Such dedifferentiation at conjugation is characteristic of the Hypotricha generally and is followed normally by a redifferentiation of the parts that were lost. The period of dedifferentiation may therefore be called for convenience the "reconstruction stage."

The most characteristic mark of this stage is an unusually large, clear, vacuole-like body, that appears in the midline toward the anterior end, and is equally visible and striking when seen from either the dorsal or ventral view. This body, called by ENGELMANN the "placenta," was believed by BÜTSCHLI (1887–1889, pp. 1624–25) to be the new macronucleus, and MAUPAS, in the work cited above, verified this interpretation. In the most recent study of the cytology of conjugation in *Euplotes patella* by TURNER (1930), the new macronucleus at this stage of its development is shown in fixed and stained specimens to correspond in size, shape, and position with the clear body in the living ex-conjugant described by the earlier workers, and observed in the present study.

This globular macronuclear anlage is probably the only clearly defined structural differentiation that persists throughout the period that is called here the reconstruction stage. From the earlier accounts and from observations on living conjugants by the present writer, the locomotor and oral appendages appear to be fully redifferentiated by the time the conjugants separate. Besides the appearance of the macronuclear anlage, the differences that define the reconstruction stage are mainly differences in size and behavior, which will be described below. Reconstruction, therefore, will be used here in the broad sense of "physiological reconstitution," the completion of which, plausibly enough, appears to await the development of the macronucleus from its embryonic globular shape to its functional horse-shoe shape. Complete "redifferentiation" will signify resumption of the vegetative condition in macronuclear structure, body size, and general behavior.

Figure 1 shows camera lucida outline sketches of ex-conjugants during the reconstruction stage and of a normal non-conjugant of the parent clone made at the same time. Unfortunately no fixing method has yet proved satisfactory for preserving the precise outlines of the living animals or any of the visible internal structures, including the arrangement of the transparent and opaque areas. These latter become conspicuously altered in the dedifferentiated ex-conjugants, a change which adds a further means

of distinguishing them from the fully developed animals. The sketches shown give an idea of the comparative size, the general shape, and the position and size of the macronucleus of new ex-conjugants, but are very inadequate representations of their actual appearance, and of the extent of the differences between them and the fully differentiated animals.

The size of new ex-conjugants is about that of young individuals at the time of separation following division, or about half that of full grown individuals just before the constrictions of fission have begun to appear. Despite their similarity in size, however, the ex-conjugant in the reconstruction stage and the youngest individual formed in fission are so different in appearance that the two are readily distinguishable at a glance, even with

FIGURE 1.—Camera lucida drawings of ex-conjugants during the reconstruction stage and of a non-conjugant. a and b, ventral and dorsal views of dedifferentiated ex-conjugants, at about the beginning of the third day after conjugation. c, a non-conjugant of the parent clone. Chrom-osmic fixation.

the lowest powers of the dissecting binocular. The period of dedifferentiation persists through the first and second days following conjugation, and during this time the ex-conjugants swim around very sluggishly and appear not to eat. Until near the end of the second day there is no apparent increase in size. But at about this time or more usually about the beginning of the third day, redifferentiation accompanied by growth begins, and during the course of the third day the normal appearance and adult size are gradually resumed, although in a few cases they may not be completed until the early part of the fourth day. Fission then begins, in most cases the animal dividing once during the day following the completion of regeneration.

These events, however, do not take place in every case. Usually in a large percentage of the ex-conjugants they do not occur at all; such individuals instead live a day or so longer, or at times as long as 10 or 12 days, become steadily weaker and thinner, and finally die without having begun to redifferentiate. In every observed case, ex-conjugants which did not redifferentiate by the fourth day never attained the adult size and structure and consequently never divided.

In the present experiment 102 non-conjugants and 102 conjugating pairs were taken on November 15, 1929 from a small mass culture known to be derived from a single individual by vegetative reproduction only. On the following day, the 102 conjugating pairs had all separated. In 15 of these, however, the two members were found to have the usual adult structure, necessitating the conclusion that they had not completed conjugation. These 15 pairs were accordingly discarded. The two members of each of the 87 remaining pairs were isolated in separate moist chambers and at the same time were named (1*a* and 1*b*, 2*a* and 2*b*, et cetera). The 102 lines of non-conjugants, each of which had divided once or twice on the day following isolation, were transferred and their daily records of fissions started.

On November 20, five days after conjugation, many of the ex-conjugants were found to be still undifferentiated and a few had already died. Of the 174 ex-conjugants with which the experiment was started, 79, about 46 percent, failed to redifferentiate, and eventually died without dividing. The number left was too small to furnish a group of adequate size for the study planned, especially since there remained only 35 complete pairs. It was necessary, therefore, either to start again with several hundred ex-conjugants or to augment the group now on hand with more ex-conjugants from the same parent clone. The latter course, appearing to be feasible, practical, and less laborious, was adopted. Accordingly, on November 21, the excess animals from the non-conjugant lines were put together in a culture dish and fresh fluid added. On the morning of November 23, the culture was found conjugating and 144 pairs were isolated as before. This group of conjugants was called the "β group" to distinguish it from the first or "α group." The non-conjugants were called the control or "C group."

The members of pairs of the β group were not isolated on the day following conjugation as in the α group, but were kept on the same slides until the majority had assumed the normal appearance, that is, for three days. On each day most of the old fluid was pipetted from each concavity and fresh fluid added. This difference in treatment was necessitated by the fact that the number of uniform slides available was limited. At the end of the three days all redifferentiated ex-conjugants were isolated and named as in the α group; those that remained undifferentiated were discarded. This procedure yielded altogether 74 complete pairs and 31 unpaired individuals, or 179 lines of ex-conjugants in the β group.

The addition of the second group of ex-conjugant lines made the total number so large that the daily routine of transferring and recording could not be managed with sufficient care and accuracy by one person. Dr. RUTH S. LYNCH very kindly consented to take charge of part of the work, and

accordingly undertook at this point the care of most of the lines in the α group and some of the non-conjugants.

Before going into the account of the data that were obtained it will be necessary first to explain how the results for the two different ex-conjugant groups were handled in relation to the single non-conjugant control group with which they are to be compared. The α group was run for 35 days and the β group for 30 days, so that neither the beginning nor the ending of the two coincide. In addition to this the records of the C (control) group started before those of either of the other two, and continued until the ending of the β group, going beyond the period of the α group. In order, therefore, to make accurate comparisons possible, the records of the C group are considered from the standpoint of two "ranges"; one of these coincides with the total period of the α group and is called the "α range," the other coincides with the total period of the β group and is called the "β range." These relations are shown diagrammatically in figure 2. As the

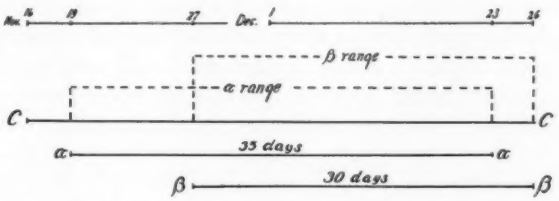

FIG. 2.—Diagram of chronological relations between the two ex-conjugant groups, α and β, and the non-conjugant group, C. See text.

diagram shows, the C data between November 19 and December 23 are compared with the α group, and the C data between November 27 and December 26 are compared with the β group.

Mortality and reproduction

In addition to the more fundamental considerations of variation and inheritance, the questions of the comparative mortality of non-conjugants and ex-conjugants, and the reproductive capacities of these two groups as shown by their gross mean fission rates, are of some interest. The data of the present investigation show that in the α group 56.1 percent of the ex-conjugant lines died out before the end of the experiment, and in the β group 38.1 percent. In both groups these mortality percentages include the ex-conjugants which failed to redifferentiate. Among the non-conjugants there was a total mortality of 6.8 percent. This large difference in mortality between ex-conjugants and non-conjugants has been

demonstrated repeatedly in Paramecium and frequently in other forms. Exceptions, however, are to be found.

An unusual set of relations between the rates of reproduction of ex-conjugants and non-conjugants is shown by the data of the present study, given in table 1. The mean daily fission rate of the α group of ex-conjugants is slightly lower than that of the non-conjugants, while the mean daily rate of the β group is slightly higher. It is not possible at present to determine the significance of this.

TABLE 1

Means of the mean daily fission rates of the lines in the two ex-conjugant groups, α and β, and in the corresponding ranges (periods) of the non-conjugant group, based on the data summarized in table 2.

GROUP	NON-CONJUGANTS	EX-CONJUGANTS	DIFFERENCE
α	$1.141 \pm .003$	$1.102 \pm .012$	$.039 \pm .013$
β	$1.124 \pm .005$	$1.154 \pm .006$	$-.030 \pm .008$
Difference	$.017 \pm .006$	$.052 \pm .013$	

Variation

Table 2 gives for each group the frequency distribution of the mean daily fission rates for all lines that lived ten days or more, and serves as an inclusive summary of the fundamental data obtained from the experimental records. The effect of conjugation on variability is obvious: the range of fission rates is very much greater after conjugation than before. This effect is all the more striking in view of the probability that an extensive selective elimination occurred among the ex-conjugants coincident with their high mortality. New low rates—from 0.2 to 0.85 fissions per day—are shown by the ex-conjugant lines; in the high rates the proportion of ex-conjugants represented is considerably greater than that of non-conjugants (see, under class 1.20, 38 β ex-conjugants to 10 non-conjugants; and under class 1.25, 12 α ex-conjugants to 3 non-conjugants and 50 β ex-conjugants to 2 non-conjugants). In addition, in the α group one ex-conjugant reaches a distinctly new maximum (1.4 fissions per day).

TABLE 2

Distribution of mean daily fission rates in the two ex-conjugant groups, α and β, and in the corresponding ranges of the non-conjugant group. The mean rates are carried to the nearest .05. Only lines that lived ten days or more are included.

MEAN DAILY RATES		.20	.25	.35	.50	.55	.70	.75	.85	.95	1.00	1.05	1.10	1.15	1.20	1.25	1.40
α	Ex-conjugant	2				2	1		1		6	4	24	27	11	12	1
	Non-conjugant										2	1	31	48	15	3	
β	Ex-conjugant		1	1	1		1	1		3	1	6	16	56	38	50	
	Non-conjugant									1	2	15	19	49	10	2	

These differences in variability are shown most conveniently by comparing the standard deviations or the coefficients of variation of non-conjugant and ex-conjugant lines. The standard deviation is a measure of the amount, within the group, of departure from the mean, expressed directly in terms of the original units—in this case, fissions per day. The coefficient of variation is the standard deviation expressed as a percentage of the mean. The standard deviations and the coefficients of variation for the two ex-conjugant groups and their corresponding ranges in the non-conjugants are shown in table 3. These are calculated from the data summarized in table 2. In the α group, the ex-conjugants show a standard deviation more than four times as great as that of the non-conjugants, .18 for the former as compared with .04 for the latter, with the coefficients of variation increasing from about 4 percent for the non-conjugants to over 16 percent for the ex-conjugants. Likewise, in the β group the ex-conjugant standard deviation is 1.5 times that of the non-conjugants, with the coefficient of variation increasing from about 7 percent to over 11 percent. The statistical significance of all these values is obvious. It must be concluded that the variability in fission rate of a group of ex-conjugants of *Euplotes patella* is much greater than the variability of the clone from which they were derived.

TABLE 3

Standard deviations and coefficients of variation of the mean daily fission rates in the two ex-conjugant groups, α and β, and in the corresponding ranges of the non-conjugant group, based on the data summarized in table 2.

	GROUP	NON-CONJUGANTS	EX-CONJUGANTS	DIFFERENCE
Standard deviation	α	.045 ± .002	.181 ± .009	.136 ± .009
	β	.086 ± .004	.130 ± .004	.044 ± .005
Coefficient of variation	α	3.96 ± .18	16.43 ± .84	12.47 ± .86
	β	7.66 ± .37	11.30 ± .41	3.64 ± .45

Inheritance

In connection with the preceding demonstration of increased variability following conjugation, there arises the important question: Are the differences among ex-conjugants genetic differences? Has conjugation actually changed the hereditary constitution of those individuals that underwent this process, so that they are now true genetic variants, among whom the differences will persist?

This question may be examined directly and simply by comparing the records of ex-conjugant lines having different fission rates, and observing whether these differences are maintained throughout a series of short consecutive periods. In table 4 are shown the records of fissions during suc-

cessive 5 day periods of three representative lines from the α group of ex-conjugants and of three from the β group, the three in each case consisting of a high, an intermediate, and a low line. Included also in the table are the total fissions of each line and its mean daily fission rate. Seven such periods are provided by the α group and six by the β group. The selection of lines here was limited to those living the full time of observation in order to give in each case a complete set of periods for comparison. Lines α34a and β10b are seen to be consistently higher than lines α31b and β17b respectively. These latter in turn are consistently higher than lines α3a and β51b respectively. In each case the differences in total fissions and mean daily fission rates are reflected for the most part in each of the successive five day periods. For these lines the differences are inherited.

TABLE 4

Fissions during successive 5 day periods, total fissions for 35 days (α group) or 30 days (β group), and mean daily fission rates of six representative ex-conjugant lines.

GROUP	LINE	5 DAY PERIODS							TOTAL FISSIONS	MEAN DAILY FISSION RATE
		1	2	3	4	5	6	7		
α	34a	7	7	6	6	6	6	6	43	1.23
	31b	6	5	5	5	4	5	5	35	1.00
	3a	4	4	3	4	4	4	1	24	0.68
β	10b	6	6	7	6	7	6		38	1.26
	17b	4	6	4	4	6	5		29	0.96
	51b	4	5	3	4	2	3		21	0.70

The same sort of relations exist generally throughout all the lines of both groups of ex-conjugants. This can be shown by the amount of correlation, for all lines of a group, between the fission rate during an early period and that during a late period in each line. The magnitude of the coefficient of correlation so obtained for each group will show to what extent the differences among the lines of that group are perpetuated, that is, will provide a measure of the degree of inheritance of fission rate within the group. Since the α group was run for 35 days, in the lines of that group and in the non-conjugants during the α range, the mean daily fission rate during the first 17 days is correlated with that during the last 18 days for each line. Similarly, in the β group and in the non-conjugants during the β range, the total period here being 30 days, the mean daily fission rate during the first 15 days is correlated with that during the last 15 days for each line. Lines that lived less than ten days during the late period are not used. The coefficients calculated for each group are shown in table 5, and the correlation tables from which the calculations are made are given as

tables 5a, 5b, 5c, and 5d, with the group which is represented indicated for each.

TABLE 5

Coefficients of correlation of mean daily fission rate in the early period with that in the late period of each line, in the two ex-conjugant groups, α and β, and in the corresponding ranges of the non-conjugant group.

GROUP	NON-CONJUGANTS	EX-CONJUGANTS	DIFFERENCE
α	.071 ± .068	.723 ± .036	.652 ± .077
β	−.086 ± .068	.558 ± .035	.644 ± .075

TABLE 5a

Correlation in mean daily fission rates between early and late periods (first 17 days and last 18 days) of lines of the α ex-conjugants.

	.75	.95	1.05	1.10	1.15	1.25	1.30	
.30	1							1
.60	1							1
.80		1						1
.90				1				1
.95			1					1
1.00				1	2			3
1.05			2	5	5	1		13
1.10			3	5	8	5		21
1.15			1	4	6	6	2	19
1.20			1	3	4	5		13
1.25					1	4		5
	2	1	8	19	26	21	2	79

r = .723 ± .036

TABLE 5b

Correlation in mean daily fission rates between early and late periods (first 17 days and last 18 days) of lines of the non-conjugants in the α range.

	1.00	1.05	1.10	1.15	1.25	1.30	
.80					1		1
.95				1	1		2
1.00	1			8	2		11
1.05			1	11	14		26
1.10		1	5	14	12	2	34
1.15			1	6	11		18
1.20				2	1	1	4
1.25				1			1
	1	1	7	43	42	3	97

r = .071 ± .068

TABLE 5c

Correlation in mean daily fission rates between early and late periods (first 15 days and last 15 days) of lines of the β ex-conjugants.

	0	8.0	.95	1.00	1.05	1.15	1.20	1.25	1.35	
.55			1							1
.60		1								1
.95			1	1						2
1.00	(1)		1		1	2				4
1.05			1		1	9	7			18
1.15				2	2	13	17	10		44
1.20				2	4	14	24	26	5	75
1.25						4	13	6		23
	(1)	1	4	5	8	42	61	42	5	168

$r = .558 \pm .035$

TABLE 5d

Correlation in mean daily fission rates between early and late periods (first 15 days and last 15 days) of lines of the non-conjugants in the β range.

	.85	1.00	1.05	1.15	1.20	1.25	1.35	
.65				1				1
.95				3				3
1.00			1	5	5	1		12
1.05			5	8	8	2		23
1.15	1	1	5	10	13	2	1	33
1.20		1	5	8	8			22
1.25			1		1			2
	1	2	17	35	35	5	1	96

$r = -.086 \pm .068$

These results are striking and appear to be conclusive. Among the non-conjugant lines the diversities cannot be demonstrated as heritable. The coefficients for the non-conjugants are very close to zero, and consequently show that the non-conjugant lines are a homogeneous group in which no persistent differences can be found. They are members of a clone in the strictest sense, having, as far as can be detected, very similar genetic constitutions. In both ex-conjugant groups, on the other hand, highly significant coefficients, in one case over .7 and in the other .5, demonstrate that the comparatively great diversities between the lines of these groups persist and so are inherited. The ex-conjugant lines behave as so many different clones, each with its characteristic fission rate, distinctly maintained, for the most part, during the period of observation.

Biparental inheritance

CALKINS (1902) and CULL (1907) brought up the question of whether there exist inherent differences, comparable to sex differences, between the members of conjugating pairs in Paramecium. They pointed out that the two lines descended from the two members of a pair frequently differ as to survival and death, and held that this may indicate that one (reproducing strongly) corresponds in some degree to the female, the other (reproducing weakly) to the male. JENNINGS, at first alone (1913) and later with LASHLEY (1913), showed however that on the average the two lines descended from the two members of a pair are more alike as to survival and rate of reproduction than are two lines selected at random. This greater similarity they consider evidence for "biparental inheritance."

The data of the present experiment provide an opportunity to examine this question. Considering first certain bits of observable data, some of the ex-conjugant lines seem to indicate clearly that the above mentioned type of biparental inheritance is a consequence of conjugation in Euplotes. In the β group of ex-conjugants the lines descended from the two members of pair 41 and the two members of pair 42 show some interesting relations in their fission rates. The lines 41a and 42a were cultivated side by side on a slide in one moist chamber, the lines 41b and 42b in the same manner in another moist chamber. Under these conditions, conceivably capable of obscuring small differences between the progeny of ex-conjugants of diverse pairs, and creating differences between the progeny of members of pairs, the records shown in table 6 were obtained. The data for the four lines show that 41a and 41b are consistently lower than 42a and 42b, and that in both cases the progeny of two members of a pair are closely similar throughout. The totals and means show these relations strikingly. In these lines there is unmistakable evidence of biparental inheritance.

TABLE 6

Records of the number of fissions in successive 5 day periods, the total fissions for 6 periods, and the mean daily fission rates of 4 representative lines of the β group of ex-conjugants.

PERIOD		1	2	3	4	5	6	TOTAL	MEAN
Fissions per 5 day period	41a	5	4	5	5	5	6	30	1.00
	41b	5	5	4	4	5	5	28	0.93
	42a	6	7	6	6	6	6	37	1.23
	42b	7	6	6	6	6	6	37	1.23

The lines descended from another pair of β ex-conjugants are of particular interest. Both members of pair 51 of this group were observed to have become changed in form after conjugation so that their appearance was

plainly different from that of typical animals (figure 3). The most striking fact about this change was that it was of exactly the same kind in both of the mates. Instead of having the usual elliptical outline form of *Euplotes patella*, these two were circular in form. During the course of vegetative reproduction the structural appearance of the lines derived from these two ex-conjugants was given particular attention and it was found that the new circular form was consistently inherited in both. The rather low fission rates of the two were likewise very similar. The number of fissions for the

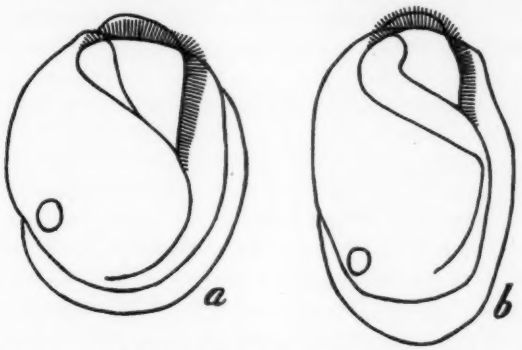

FIGURE 3.—Camera lucida drawings of a circular adult, a, and a normal elliptical adult, b. Iodin fixation.

two during successive five day periods, together with the record of a typical elliptical line of average rate, β49b, shown for comparison, are given in table 7.

TABLE 7

Records of the number of fissions in successive 5 day periods, with the total for 6 periods and the mean daily rates, in the two "circular" lines derived from pair β51, as compared with the fissions of an average normal elliptical line, β49b.

PERIOD		1	2	3	4	5	6	TOTAL	MEAN
Fissions per 5 day period	β51a	4	5	3	4	2	3	21	0.70
	β51b	4	5	5	4	4	0	22	0.73
	β49b	6	6	6	6	6	5	35	1.16

It should be mentioned that throughout the period of observation, starting before the new form was detected, that is, before redifferentiation, the two lines derived from pair β51 were run in different moist chambers so that the animals and the record slips of each were handled entirely independently of each other. The progeny of the two members of the pair are seen not only to differ by similar amounts from the fission rate of the av-

erage line, but both their similarity to each other and their difference from the other line are maintained for the most part from period to period. Small mass cultures of these two lines of circulars were started during the experiment and kept for further study. They were observed closely for several months—given daily attention and subcultured at intervals. During this time one of them, the 51b culture, was studied experimentally to determine the results of conjugation within it. An account of that work will be presented in a paper to follow. The point that is of interest here is that the circular form persisted through vegetative reproduction; in the lines and mass cultures no reversions to the typical elliptical form were detected.

The relations indicated by the foregoing cases are borne out generally in a statistical examination of the β group of ex-conjugants, though in the α group the relations were not clear. When the daily fission rates of the lines derived from the two members of each of the 69 pairs of the β group are correlated, there is found a coefficient of correlation of $.649 \pm .033$. As shown by the small probable error, this coefficient is significant; it shows a high degree of correlated variability in the two lines descended from the two members of the pairs. But in the 31 pairs of the α group no significant correlation appeared, the coefficient being $.041 \pm .084$. It is possible that in the α group the number of pairs (31) was too small to adequately test the matter; here the probable error is larger than the coefficient obtained. The matter needs further examination.

DISCUSSION AND SUMMARY

The foregoing account has demonstrated that in *Euplotes patella* the effects of conjugation are in general similar to those that have been found in Paramecium by JENNINGS (1913) and RAFFEL (1930). Before conjugation the members of a clone have a comparatively small range of fission rates with, moreover, no consistent maintenance of the limited number of diversities that can be demonstrated. After conjugation the ex-conjugants give rise to lines whose fission rates cover a wide range, and among whom the comparatively large number of diversities are consistently perpetuated. On the basis of these facts it may be concluded that in *Euplotes patella*, as in Paramecium, conjugation breaks up a single clone into many new clones.

The detailed results of the investigation may be summarized as follows:

1. Coincident with conjugation, *Euplotes patella* goes through a stage of dedifferentiation, called the "reconstruction stage," during which their appearance is strikingly different from that of non-conjugants and older ex-conjugants. Reproduction is discontinued during this period, which

lasts from two to four days and is followed usually by redifferentiation, with resumption of the normal structure and functions. In a large proportion of cases, however, redifferentiation does not take place, the animals passing directly from this stage to death without reproducing. In no case was redifferentiation found to take place after the fourth day following conjugation.

2. The mortality among ex-conjugants is from five to eight times as great as that among the parent non-conjugants.

3. The mean fission rate of ex-conjugant lines differs little from that of the parent non-conjugant lines. In one case it was slightly higher, in another slightly lower.

4. Ex-conjugant lines are considerably more variable in fission rate than their parent non-conjugant lines.

5. The small differences which occur among the non-conjugant lines of a single clone are not inherited; the large differences among the lines derived from conjugants of the clone are inherited to a very marked extent.

6. In one group of ex-conjugants individual cases and statistical relations indicate a high degree of similarity between the progeny of members of pairs. In the other smaller group no such similarity can be demonstrated statistically.

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ON THE INHERITANCE OF BODY FORM AND OF CERTAIN OTHER CHARACTERISTICS, IN THE CONJUGATION OF EUPLOTES PATELLA

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INTRODUCTION

The present paper is an account of results obtained from conjugations within a clone of *Euplotes patella* of atypical form, and within clones descended from some of the ex-conjugants produced. In the foregoing paper (COHEN 1934) on the effects of conjugation in this hypotrichous ciliate a study was made of a group of conjugants obtained by inducing conjugation among the members of a single clone of typical elliptical form. The two members of one pair of conjugants, pair β51b, were found after separating to have become altered from the elliptical to a circular form. This form was assumed, as far as could be detected, as soon as the animals had completed the changes of the reconstruction stage which occurs at conjugation. (See the foregoing paper for an account of these changes during the reconstruction stage.) Camera lucida drawings of a "circular" and a typical elliptical animal are reproduced here in figure 1 to show the difference in form between the two.

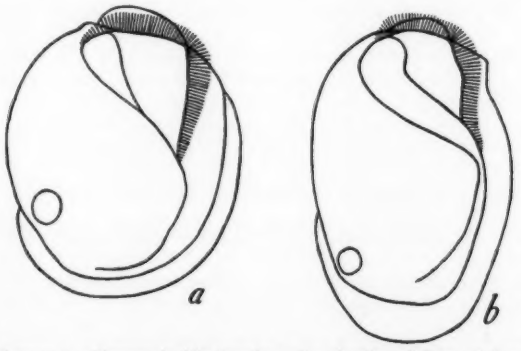

FIGURE 1.—Camera lucida drawings of a circular adult, a, and an elliptical adult, b. Iodin fixation.

The essential facts about the origin, early treatment, permanence, and characteristics of the structurally peculiar individuals may be summarized as follows:

1. The new form appeared in only 2 ex-conjugants (one pair) out of 271. It was recognized in each only after the two had been isolated in separate moist chambers.

2. The low frequency seems to indicate a mutation coincident with conjugation rather than the selecting out of already existing factors. However, a frequency of this order could result from a dependence of the characteristic on multiple factors, and since in the present case the term "mutation" has no further factual meaning than simply "low frequency," it will probably be best to regard the appearance of the new characteristic only as a result of conjugation.

3. The peculiarity was inherited consistently through vegetative reproduction during 30 days of observation of the isolated lines. The two lines derived from the two members of the pair were in different moist chambers throughout this time and were handled, and their records made, independently of each other.

4. In addition to the diversity in form, the individuals of the two lines differed also from the normal elliptical in their gross internal structure. The difference consisted essentially of the slight distortion of a clearly delimited area which appears opaque with transmitted light. This distortion may be characterized as what would occur if an elliptical animal were slightly compressed mechanically in an antero-posterior direction.

5. The fission rates of the two circular lines were very similar and unusually low. For one the mean daily rate was .70 fissions per day and for the other .73 fissions per day. Out of the total of 175 lines whose fission rates were determined, only three had rates lower than these two, and since the mean of the group was 1.12 fissions per day, these two were rather extreme variants. Considering the independence in culturing, the similarity in fission rate must have been due to genetic likeness.

6. Mass cultures of the two races, started with individuals from the isolation lines, were maintained as pure clones by daily attention. These showed no reversions to the elliptical type.

The circular form in this species has previously been found in nature. SAVILLE KENT (1882), in his description of *Euplotes patella*, says (p. 798): "This species, like many other Hypotricha, is subject to considerable local and individual variation, chiefly evinced in this instance in the varying contour of the carapace, and in the character of its ornamentation. This carapace or cuirass, while normally oval or elliptical, may be nearly circular or lozenge-shaped, broad in the center and much narrower at the antero-posterior extremities." From such an account, of course, it cannot be learned if such variants are distinct clones, as is true in the present case, but it is possible that they are, and that we have in the present case a

direct observation on the probable means by which some of the diversities found in nature come to exist, namely, as a result of conjugation.

No record has been found of any other case essentially similar to the one under consideration here. The unique features of this characteristic are its origin during conjugation, its affecting the descendants of both conjugants, its persistence through vegetative reproduction, and as will be shown later, its mode of inheritance through subsequent conjugations. Morphological diversities that persist through vegetative reproduction have been demonstrated before in protozoa, but with no great frequency considering the amount of work that has been done on the genetics of these forms. They have been described by JENNINGS (1913) and STOCKING (1915) in *Paramecium caudatum*, by DAWSON (1926) and MOORE (1927) in *Paramecium aurelia*, by REYNOLDS (1923) and JOLLOS (1924) in the rhizopod Arcella, and by MACDOUGALL (1931, 1931a) in Chilodon.

However, even in these comparatively few descriptions, it is only in respect to the one feature of permanence of the visible character through successive fissions that all of them are similar to what has been observed in Euplotes. In regard to the origin of the diversities, in only three accounts is this known at all; in two of these it appears that the characteristic arose as in the present case during conjugation. This last is shown by JENNINGS and by STOCKING in *Paramecium caudatum*. JENNINGS observed that after conjugation the lines from some ex-conjugants showed a persistent tendency to produce abnormal individuals of indefinite and usually bizarre shape. This was evidenced with varying frequency in diverse lines. STOCKING made an intensive study of this, working out in detail and establishing the conclusions of JENNINGS, that as a result of conjugation some lines acquired a genetically permanent tendency to become abnormal and this was expressed in different degrees in various lines. However, while such abnormalities are indeed structural, they differ from the peculiarity in Euplotes in their inconstancy of manifestation.

The third case in which the origin of the diversity is known is MACDOUGALL'S recent work on the production of a structural peculiarity by ultra-violet radiation in *Chilodon uncinatus*. Normal specimens of Chilodon, when radiated, were found to give rise to mutated forms which, in addition to less conspicuous structural changes, possessed a clearly discernable posterior prolongation, which she calls a "tail." It was shown in this work that inbreeding of the tailed forms resulted in the production anew of both normal and tailed individuals. In addition the other less conspicuous characters, which consist of a new form and new ciliation, may in some inbreedings be segregated from "tailed" and thereafter be inherited independently. In other inbreedings these characters are not separated but remain together consistently. MACDOUGALL has also twice

succeeded in crossing diverse clones of Chilodon. In one such cross-conjugation between a homozygous tailed clone and a normal clone none of the conjugants survived. Another between the heterozygous tailed form and the normal was successful but the results have not yet been published.

Besides this work of MACDOUGALL only one other paper, that of DAWSON, discusses the behavior of the structural diversity in conjugation. DAWSON found that the character he studied persisted after the animals possessing it had conjugated together.

The explanation of such phenomena as those observed in Chilodon and, as will be shown, in Euplotes, seems to involve the kind of genetic mechanism that has been so extensively demonstrated in the metazoa. It is known that in many species of Protozoa nuclear processes occur at conjugation that are in general similar to those involved in the formation of gametes and zygotes in higher forms. There is likewise reason to believe that protozoan chromosomes, at least in some genera of ciliates, are not essentially different in structure or behavior from those of the well-studied higher forms. As far as behavior is concerned, there is much evidence that they are similar. In a recent work by TURNER (1930) on the cytology of division and conjugation in *Euplotes patella*, in which he describes the chromosomes of this species, he shows the diploid number to be eight, and in pronucleus formation at conjugation, he finds that these are reduced quite conventionally to four. Subsequently, after the exchange of pronuclei between the members of the conjugating pair, fusion of the migratory and stationary pronuclei occurs in each with restoration of the chromosome number to eight. Similar meiotic phenomena involving definite chromosomes have been described in many other protozoa. However, no exact relation has as yet been demonstrated between the chromosomal behavior in protozoa and what is known of their genetics. A suggestive approach has been made by PASCHER who described (1916, 1918) the production of clear cut intermediate types by crossing two different species of the flagellate Chlamydomonas. Union of two gametes, one from each of the species, resulted in a cyst which was intermediate in form between the cysts of the two species formed by inbreeding. In Chlamydomonas swarm spores are produced in the cyst by fissions in which chromosome reduction occurs. When formed from the hybrid cysts, these swarm spores were found in some cases to be again like those of the parents, in other cases to consist of four types, having four diverse combinations of the characteristics of the parents.

The present investigation was carried on under the direction of Professor H. S. JENNINGS, for whose interest in the work and generous assistance in the preparation of this paper the author wishes to record his appreci-

ation. He is likewise indebted to Dr. TRACY M. SONNEBORN and Dr. RUTH S. LYNCH for valuable suggestions and criticisms in the writing of the paper.

MATERIALS AND METHODS

The culture methods and general technique used in the present work are essentially the same as those employed in the experiment described in the foregoing paper. Included there are descriptions of culture fluid, methods of transferring, glassware, et cetera, so that these details need not be repeated here. The major consideration in the work to be described here is the form of the individuals of various clones. The method used in determining this was simply by recording from day to day, at the time the animals were transferred, the result of visual observation of the outline form of the individuals on the slides. For this the low power (X20) of the binocular dissecting microscope, which was used in all transferring and general routine treatment of the animals, was adequate. The condition observed was indicated by symbols placed alongside the record of the fissions for the preceding twenty-four hours.

All the work to be described was done with the clone descended from one member of the pair of circulars discussed in the introduction. This was the individual known as 51b. In figure 2 is shown diagrammatically a general

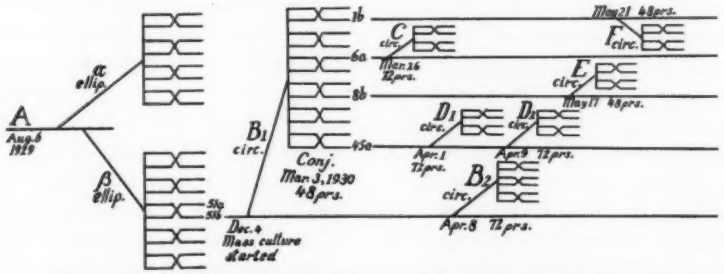

FIGURE 2.—Diagram showing the derivation of the different clones and groups of conjugants studied. For explanation see text.

plan of the origin and history of this clone and others, subsequently derived from it by conjugations, which were subjected to experimentation. In this diagram a horizontal line represents a clone, either as an isolation line or as a mass culture. A cross interrupting two such lines and joining them indicates the intervention of conjugation. An oblique line leading from a horizontal line to a group of additional horizontal lines indicates a group of animals, taken from the clone, in which conjugation was studied. As was mentioned in the introduction the first circulars arose after conjugation within a typical elliptical clone. This, clone A, arose from a single

individual isolated August 6, 1929. The conjugation in which the circulars were produced occurred on November 29, 1929; and on December 4 the clone which was used in the present work was started from an isolation line of the experiment in progress at that time.

The first group of conjugants to be examined, B1, arose March 30, 1930 from clone 51b. Of these, 48 pairs were taken for study. In addition to this the clone was maintained as such by continuing a culture of non-conjugants, and on April 8, as shown in the figure, it yielded another group of ex-conjugants, B2, of which 72 pairs were studied. In the meantime clones derived from four ex-conjugants of group B1, ex-conjugants 1b, 6a, 8b, and 45a, had been isolated as mass cultures from the respective isolation lines of that group and each of these yielded new "F2" groups of conjugants which were subsequently studied. From clone 6a arose on March 6 group C, of which 72 pairs were isolated for study; from clone 45a arose on April 1 group D1 and on April 9 group D2, in each of which 72 pairs were studied; clone 8b yielded group E on May 17 and clone 1b group F on May 21, from each of which 48 pairs were taken. The detailed treatment of each of these various groups and the experimental data obtained will be discussed in the next section. In following that discussion, reference to figure 2 will aid in making clear the derivations of the different groups and their relation to each other.

EXPERIMENTAL RESULTS

The Inheritance of Form

a. Group B1

As stated above, this group comprised 48 pairs that were isolated on March 3, 1930 from a culture of the circular clone 51b that was found conjugating on that day. Of these, four pairs had not separated by the following day so that the two members of but 44 pairs could be isolated. Of the four pairs which remained attached, separation later failed to occur, and they died in this condition within a few days. One member of one of these pairs, however, before dying and while attached to its mate, gave rise by fission to an individual which continued to live and reproduce. (This individual, which received the designation 45a and is seen thus to be the progenitor of one of the clones in which conjugation was studied later, is not of particular interest here aside from its being derived from an attached pair. In this respect it differs from the other ex-conjugants. It will be discussed in detail later in the accounts of groups D1 and D2.) Of the 89 animals which were thus obtained, 54 failed to develop from the dedifferentiated stage which normally follows conjugation, and died in this condition within six to nine days. The others were all seen to have rediffer-

entiated by the fourth day following conjugation (March 7) and on the following day most had begun to divide.

On the twenty-ninth day after conjugation all but two of the isolation lines were found dead. This wholesale mortality was clearly accidental since mass cultures previously started from some of the lines that died continued to live in good condition. The cause could not be determined. However, the data acquired showed some interesting indications.

The fact of greatest interest that appeared was that conjugation in this circular clone resulted in the production of three types of morphologically diverse lines: typical ellipticals, circulars, and intermediates. Specifically, of the lines derived from the 35 ex-conjugants which became redifferentiated:

15 were elliptical
12 were circular
8 were intermediate

This classification was made on the basis of the daily observations described in the preceding section. As shown by the actual records, the elliptical class included only lines that were distinctly and consistently elliptical, and the circular class only those which were distinctly and consistently circular; as such, both were clear-cut. The intermediate class, however, includes mainly lines whose form on most days was doubtful, in some lines being not clearly elliptical, in others not clearly circular. However, a few lines of this class showed consistently a form intermediate between elliptical and circular. But the frequent difficulty of distinguishing between these different types of intermediates forced the inclusion of all of them into a single class. The terms elliptical, circular, and intermediate will be used hereafter precisely as here defined. Of the 35 lines, 14 became thin and weak and died out before the later accidental deaths of the others. They included lines of each type. This fact will be discussed in the section on the relation of fission rate to form.

b. Group B2

On April 8, 1930, a mass culture of clone 51b was again found conjugating. This time 72 pairs were isolated to form group B2. On the following day the members of all pairs had separated, but they were not isolated at once. On April 12, four days after conjugation, 68 of the 144 individuals had become redifferentiated, and only these were transferred to fresh slides. The others were retained, however, and their deaths as still undifferentiated individuals was subsequently witnessed. The 68 fully redifferentiated animals were allowed to proliferate on the slides for four days without transferring, with the addition of fresh fluid at intervals. On the fourth

day, April 16, the forms of the animals of each line were recorded, and two to six, in most cases four, individuals were transferred to a fresh slide. This procedure was repeated two days later, on April 18, and four days later, on April 20. In this way three separate recordings of the forms of the different lines were obtained.

Apparently because of the unsatisfactory features of this type of treatment, although the specific reasons are unknown, 33 of the lines were weakened by the time the observations were made, to the extent that their form could not be determined. During the course of the observations 7 of these died. Of the remaining 35:

16 were elliptical
10 were circular
9 were intermediate

In addition to these two "F1" groups, two "F2" progeny groups, E and F, furnished data on the inheritance of form. E was derived from the circular clone 8b, and F from the circular clone 1b, both of which were descended from ex-conjugants of group B1. The remaining F2 groups, C, D1, and D2, did not provide data for this particular question.

c. Group E

Clone 8b was found conjugating on May 17 and 48 pairs were isolated on slides. All had separated by the following day, and on May 21, four days after conjugation, the redifferentiated individuals, consisting of 18 pairs and 12 singles whose mates had died, 48 animals in all, were transferred to fresh slides. These were then allowed to multiply on the slides for five days, and during this time they were observed daily and several times a drop of fresh culture fluid was added to each concavity. At the end of this period, on May 26, the form of the animals of each line was observed and recorded. As in the previous experiments no appreciable variation in form was seen among the individuals of any one line. A representative of each living line was then transferred to a fresh slide and after two days the form of the individuals of each line was again recorded. In those cases in which the records agreed perfectly as to whether the individuals of a line were clearly elliptical or clearly circular the line was classified accordingly. In a few cases where, in the first recording the form was uncertain while in the second it was clearly circular, these lines were classed as circular. All others which were uncertain in either record were classed as intermediate. There were four lines whose bad condition, resulting in a thin, pale and weak appearance, did not permit classification. Of the 44 remaining:

15 were elliptical
16 were circular
13 were intermediate

d. Group F

On May 21 clone 1b was found conjugating and 48 pairs were isolated. This group received the same treatment as group E, except that most of the redifferentiated individuals, of which there were 43, were put on fresh slides, separating the members of pairs, late on the third day following conjugation when redifferentiation could first be clearly detected. The remaining redifferentiated animals were not removed to fresh slides until the fifth day. (Their development was not certain until the fourth day). The lines of F were allowed to multiply for four days without transferring, with daily observation and addition of fresh culture fluid at intervals. The day of the first recording for group F, May 28, was the same as that of the second recording of group E. The second recording of F was on May 30. In the latter group 15 lines, as compared with 4 of E, did not permit of classification because of their weak appearance. Of the remaining 28 lines:

11 were elliptical
8 were circular
9 were intermediate

e. Summary: a–d

The results so far discussed are summarized in table 1 which gives also the percentage in each class of the total number of lines classified in each group. These percentages give some suggestion of uniformity of ratio in the production of each type of form. The possibility of interpreting the results in terms of Mendelian factors at once suggests itself, but this will be left to the concluding discussion, after all relevant data have been presented.

TABLE 1

Number of lines in each class and their percentage of the total number classified, in each group, in the total for four groups, in the two F1 groups, and in the two F2 groups.

GROUP	GENETIC RELATIONSHIP	NUMBER LINES CLASSIFIED	ELLIPTICAL		CIRCULAR		INTERMEDIATE	
			NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT
B1	F1	35	15	42.8	12	34.2	8	22.8
B2	F1	35	16	45.7	10	28.5	9	25.7
E	F2	44	15	34.0	16	36.3	13	29.5
F	F2	28	11	39.2	8	28.5	9	32.1
Total		142	57	40.1	46	32.3	39	27.4
Total F1		70	31	44.2	22	31.4	17	24.2
Total F2		72	26	36.1	24	33.3	22	30.5

The most important fact for the present is that conjugation within a clone of circulars results in the production of ellipticals, circulars, and in-

termediates, and that this result is repeated when an F2 circular clone conjugates. This is unmistakable evidence that the different forms under consideration are inherited through conjugation.

f. Similarity and dissimilarity of the descendants of the two members of a pair of conjugants

In the experiments just described, the two original conjugants were in every case circulars, but after conjugation, as we have seen, the descendants were in some cases circular, in others normal elliptical, in others intermediate—all the descendants of any one ex-conjugant being of the same type. The question arises: are the two lines descended from the two members of the same pair of conjugants always of the same type?

This question is answered in table 2. There were altogether, in the four groups B1, B2, E, and F of figure 2, 46 pairs in which the form could be determined for both members of the pairs. As shown in table 2 the two lines descended from the two members of a pair are not always of the same type, though in many cases they are. Of the 46 pairs, in 34 the descendants of the two members were alike in form while in 12 they were diverse. Table 2 shows for the four groups above mentioned the distribution of the 92 lines derived from the 46 pairs with relation to the three types of form, with the numbers and percentages in each group of the three types of form. On the whole 73.9 percent of the pairs had progeny of like type, while 26.1 percent had descendant lines of unlike types.

TABLE 2

Number and percentage of complete pairs in which the two members were alike and the two were unlike, the distribution of the pairs as to kind of likeness and unlikeness, in each group, and the totals of these for four groups. E= normal elliptical; C= circular; I=intermediate.

GROUP	NUMBER COMPLETE PAIRS	LIKE PAIRS					UNLIKE PAIRS				
		NUMBER	PERCENT	E-E	C-C	I-I	NUMBER	PERCENT	E-I	C-I	E-C
B1	12	6	50.0	4	2	0	6	50.0	4	2	0
B2	10	9	90.0	5	2	2	1	10.0	0	1	0
E	16	13	81.2	6	3	4	3	18.8	1	2	0
F	8	6	75.0	3	0	3	2	25.0	0	1	1
Total	46	34	73.9	18	7	9	12	26.1	5	6	1

Is any general tendency shown toward likeness or toward unlikeness of type between the descendants of the two members of a pair? JENNINGS (1913) and JENNINGS and LASHLEY (1913), working with Paramecium, and COHEN (1934) with Euplotes, showed that the descendants of the two members of a given pair are on the whole more alike in fission rate than are

lines paired at random. In most of these cases the conjugation was within a single clone. JENNINGS and LASHLEY (1913) and STOCKING (1915) showed a similar relation with respect to death after conjugation and with respect to abnormalities. The two lines descended from the two members of a pair have the same fate more frequently than would be given by random pairing of the ex-conjugant lines. JENNINGS and LASHLEY (1913) consider this greater resemblance in fate to indicate that both the descendant lines are influenced by the contributions from both members of the pair of conjugants; so that they spoke of it as "biparental inheritance."

What is the situation as to this in respect to form in Euplotes? This may be determined by comparing the proportions of like and unlike pairs in table 2 with what would be found in case the lines were paired at random. Such a comparison involves the following:

The 46 pairs of table 2 give rise of course to 92 ex-conjugant lines. Of these, as may readily be deduced from the table, 42 are normal elliptical, 21 are circular, and 29 are intermediate. The proportions 42E:21C:29I are almost exactly as 4E:2C:3I. Assuming this latter set of ratios, the results of pairing the lines at random are readily calculated (essentially by squaring 4E:2C:3I). This gives the following proportions:

$$16EE+4CC+9II+16EC+24EI+12CI=81$$

Reducing these proportions to percentages and comparing these calculated percentages to those actually found, we have the relations shown in table 2a.

TABLE 2A

Percentages of diverse types of pairs of lines derived from ex-conjugants, as compared with those calculated from random mating.

CONSTITUTION OF PAIRED LINES	CALCULATED PERCENT	FOUND PERCENT
E E	19.75	39.13
C C	4.94	15.22
I I	11.11	19.57
Total like pairs	35.80	73.92
E I	29.63	10.87
C I	14.81	13.04
E C	19.75	2.18
Total unlike pairs	64.19	26.09

As table 2a shows, in all cases the proportion of pairs in which the two members of a pair are alike is much greater than would be the result from random mating, while the proportion of pairs in which the two are unlike is in every case less than would result from random mating. From random mating 35.8 percent of the pairs should show like members; actually 73.9

percent show like members. From random mating, 64.2 percent would be unlike; actually but 26.1 percent have unlike members.

The evidence is strong therefore that in consequence of conjugation there is a tendency for the descendants of the two members of a given pair to be alike in form. As to the genetic processes to which this is due little that is specific can be said. The general question is discussed in a later section.

Viability

The viability after conjugation shows in these clones of Euplotes certain phenomena that are of interest. The best measure of viability is perhaps the proportion of the ex-conjugant lines that succeed in redifferentiating during the reconstruction stage. Usually, as will be seen, a considerable number of lines fail to redifferentiate and therefore die out. In certain clones all or nearly all thus fail to redifferentiate; this it was that made it seem worth while to bring together the facts on viability after conjugation.

The statistics as to viability for most of the diverse ex-conjugant groups shown in figure 2 are collected in table 3. In this table the groups C, D1, and D2 are omitted, being reserved for special consideration later.

TABLE 3

Number and percentage of lines redifferentiated, of the total number of lines in two P1, two F1, and two F2 groups, showing the relative viabilities.

GROUP	Aα	Aβ	B1	B2	E	F
Genetic relationship	P1	P1	F1	F1	F2	F2
Total number ex-conjugants	174	273	96	144	96	96
Number redifferentiated	94	177	61	76	48	43
Percent redifferentiated	54.0	64.8	63.6	52.8	50.0	44.8

As can be seen, in the six ex-conjugant groups of table 3 the proportion that redifferentiated after conjugation varied from 44.8 percent to 64.8 percent. Thus the proportion that were inviable was rather high, varying from 35.2 percent up to 55.2 percent.

In the three remaining groups, C, D1, and D2 (figure 2), all of the ex-conjugants were inviable, none of them redifferentiating. In group C, of the 72 conjugating pairs that were isolated, all members of the 144 ex-conjugants were dead 7 days after conjugation, while the non-conjugants continued to live in mass culture.

Group D1

The groups D1 and D2 are of interest from the fact that they were derived from the clone produced by the individual 45a, which individual

arose from the division of one member of a pair of conjugants that never separated. This division took place 11 days after conjugation. This, and the fact that the individual produced was at first weak and did not divide for two days, indicate that the nuclear processes of conjugation had taken place.

In group D1 conjugation occurred on April 1, and as in group C, 72 pairs were isolated. At the same time, as usual, a mass culture of non-conjugants was started; and also in this case a mass culture of conjugating pairs.

Of the conjugants on slides, all but 13 were dead on the day following conjugation (11 single individuals and both members of one pair surviving). To test whether the 60 slide concavities in which ex-conjugants had died might carry injurious conditions, a single non-conjugant was placed in each of these, and this was later repeated as other ex-conjugants died. The non-conjugants flourished, there being but 6 deaths out of the total of 71. It is clear therefore that the death of the ex-conjugants was not due to the slides.

Of the 13 remaining ex-conjugants, all but two were dead the next day (April 3). The two remaining ones later died.

In the mass culture of ex-conjugants, all were dead on April 3 except 4. Some of these four remained alive until April 12, when the last ones died. In the meantime the mass culture of non-conjugants flourished. They were found to be in conjugation on April 9, producing the conjugant group D2 of figure 2.

It is clear from this experiment that the animals were made by conjugation incapable of living in an environment that would have supported them had conjugation not intervened. It seems probable that this was the case also in group C, before described, though here the matter was not so critically tested.

Group D2

The matter was further tested with the group D2. This was derived by conjugation on April 9 from the same clone from which had come D1 (see figure 2). Again 72 conjugant pairs were isolated; also mass cultures were made, one with 100 conjugating pairs, the other with non-conjugants. On the 13th, four days after conjugation, it was found that in both the isolation lines and the ex-conjugant mass culture most of the pairs had not separated. Those that had were still undifferentiated, and most of these were weak. By the 20th all the animals in the mass culture had died, the attached pairs without separating. Among the isolation lines many had died, and by the 23rd only two attached pairs remained alive. By the 26th these had died. Throughout this time the non-conjugant mass culture con-

tinued to live and reproduce, remaining in good condition. Thus the inviability of this clone after conjugation, at least in the environment provided, is well established.

It is therefore clear that in some cases conjugation results in inviability of all the ex-conjugants, and this under conditions in which the non-conjugants flourish. It is of interest that the two groups D1 and D2, in which there was 100 percent inviability, were derived from the same clone, which itself arose from division of a member of a pair in which the two members never separated. This suggests that there was some genetic peculiarity in this clone, giving rise to the total inviability after conjugation. The group C, likewise 100 percent inviable after conjugation, arose however from another individual (see figure 2).

One other fact in connection with these experiments seems worth pointing out. The clone 45a, which arose from a pair of conjugants that died without separating, in a subsequent conjugation (D2) produced conjugants in which this tendency to remain attached again appeared. In the D1 group the lethal effect of conjugation was produced so quickly that there was no opportunity for observing whether the tendency to remain attached occurred here also. But in D2 it was manifested to a great extent, in most of the pairs, and this seems good evidence that the failure of pairs to separate is an inherited tendency.

Fission Rate in Relation to Form

The records on fission rate show that there is a relation between this characteristic and the form of the body; a relation that may perhaps be characterized as linkage. The data on these matters are drawn mainly from the study of group B1 (figure 2), which was examined more fully than the other groups.

In B1 the ex-conjugant lines were followed in detail for 27 days. In table 4 are shown for the three types of form, the number of fissions for three successive nine day periods in each of the 35 lines that redifferentiated after conjugation, together with the mean daily fission rate for each line. (In the case of lines that died out, the fission rate is based on the number of days during which the lines continued to divide.)

Table 4 shows that there was a marked difference between the fission rates in the normal elliptical group and those in the other two groups. In the elliptical group the fission rates are on the whole much higher. This is well shown in the averages for the three forms, given in table 5. The average rate for the ellipticals is double that of the others. The slight difference between the means for the circulars and intermediates (.42 and .47) is hardly significant in view of the small numbers; these two groups have about the same mean fission rate.

TABLE 4

Number of fissions for three 9 day periods, for the total of 27 days, and the mean daily fission rate, of the individual elliptical, circular, and intermediate lines of group B1.

CHARACTERISTIC	LINE	9 DAY PERIOD			TOTAL FISSIONS	NUMBER OF DAYS OF FISSION	MEAN DAILY FISSION RATE
		1	2	3			
Elliptical	2a	5	12	10	27	27	1.00
	4a	6	11	10	27	27	1.00
	5a	6	11	10	27	27	1.00
	5b	6	7	x	13	18	.72
	9b	6	10	11	27	27	1.00
	10a	6	11	10	27	27	1.00
	11a	6	12	10	28	27	1.04
	11b	5	12	11	28	27	1.04
	12b	6	11	11	28	27	1.04
	13a	6	11	11	28	27	1.04
	13b	6	11	11	28	27	1.04
	16a	5	11	10	26	27	.96
	33a	6	11	10	27	27	1.00
	33b	2	x		2	8	.25
	43a	6	11	11	28	27	1.04
Circular	1a	2	x		2	7	.29
	1b	4	8	6	18	31+	.58
	3a	1	x		1	6	.17
	6a	5	5	3	13	27	.48
	7a	2	x		2	7	.29
	7b	5	8	5	18	27	.67
	8b	5	7	1	13	27	.48
	21a	2	x		2	7	.29
	22b	3	4	2	9	27	.33
	30a	2	x		2	7	.29
	34b	4	5	3	12	27	.44
	45a	0	9	9	18	23	.78
Intermediate	2b	4	3	x	7	13	.54
	6b	5	10	10	25	27	.93
	8a	1x			1	6	.17
	9a	3	3	x	6	14	.43
	10b	2	x		2	8	.25
	12a	5	9	4	18	31+	.58
	18a	4	1	x	5	10	.50
	36b	2x			2	6	.33

TABLE 5

Number and percentage of lines viable, and the means of the mean daily fission rates, for the total elliptical, circular, and intermediate lines of group B1, based on the data of table 4.

CHARACTERISTIC	NUMBER OF LINES	NUMBER OF LINES VIABLE	PERCENT VIABLE	MEAN DAILY FISSION RATE
Elliptical	15	13	86.6	.94
Circular	12	4	33.3	.42
Intermediate	8	4	50.0	.47

Within each group there are variations in the fission rate, and some of these appear of much interest. The two low lines (5b and 33b) of the elliptical group are hardly significant, since these lived but a few days. But in the intermediate group one line (6b) has a rate (.93) practically double that of the average for this group, and very near to the average rate for the elliptical group. Also, one of the lines of the circular group (45a) has a rate of .78, while another (34b) that lived throughout the entire period has a rate of but .26, or just one-third that of the high line 45a. These facts indicate that low and high rates may not be indissolubly linked to circular and elliptical forms respectively.

As seen in table 4, the proportion of lines that lived the full 27 days of observation varies in the three form groups. In all the lines which died out before the end of this period there was a distinct "running down," marked by, first, weakness of the animals, then the cessation of division, and finally a more or less protracted wasting away followed by death. Those lines which escaped this running down may be spoken of as "viable" in comparison to the others, although this viability is clearly different from that discussed in the preceding section. Table 5 gives the percentage of such viable lines in the three form groups. As was true for fission rate, the normal elliptical lines have far the highest viability.

DISCUSSION

Conjugation within a clone of protozoa has been regarded, on the basis of the known cytological facts, as showing the essential characteristics of inbreeding, or perhaps more specifically self-fertilization, as the term is used in Mendelian genetics to denote mating between gametes arising vegetatively from the same original fertilized egg. Inbreeding or self-fertilization, however, involves fundamentally the germinal materials that are the basic determining factors in all types of mating: their arrangement and segregation in the parents; their distribution to the progeny; their rearrangement and resegregation in the progeny. In biparentally reproducing metozoa and metaphyta, these things are well understood, and the way in which they occur has been formulated to a degree which permits the coordination of most of the observable facts into a closely integrated scheme, and warrants the prediction and verification of additional facts. But in the protozoa they are not so well understood, and it still remains to be proved that inbreeding in these forms actually involves true Mendelian factors.

It is true, however, that many of the facts that have appeared in the study of conjugation in protozoa at least indicate this. In the production of new biotypes by conjugation, for example, the suggested interpretation is that factors which determine the characteristics of the new biotypes become segregated and then recombined in new arrangements during the

formation, exchange, and fusion of pronuclei at conjugation. But in order to prove that such segregation and recombination is of the Mendelian type, it is necessary to study diverse characteristics which are sufficiently contrasted, so that the behavior of the determining factors is sharply reflected in the characteristics, and to obtain them after conjugation in definite ratios that are constant. It is the lack of this type of result that has tended to obscure the essential significance of mating in protozoa, as regards the behavior of genetic factors. Fission rate, viability, and size, although their study has yielded much valuable information, do not present sufficiently contrasted differences or definite enough ratios to permit an examination of the precise behavior of the factors which determine them.

The experimental facts that have been demonstrated in the present account, which is concerned with inbreeding in morphologically distinct clones and with the inheritance of clearly contrasted characteristics, may throw some light on the problem. As pointed out in the introduction, MACDOUGALL has already made a step in this direction. She has shown so far that by conjugation individuals having well defined characteristics of diverse types can arise from a clone in which the parent individuals have these characteristics either combined or not expressed.

In the present case it has been shown that when a clone of circulars derived from normal ellipticals is inbred the progeny may be either elliptical, circular, or intermediate. In Mendelian genetics this is one of the most familiar of the types of results obtained in breeding experiments. It seems probable that the circular parents are heterozygous, in the sense of carrying both dominant and recessive factors that determine form. The simplest type of factor combination that can produce this result by inbreeding is a single pair, represented in each parent by a dominant and a recessive factor. However, the ratio obtained from such a type of mating, apparently one homozygous dominant to two heterozygous to one homozygous recessive, does not at all compare with the ratios obtained from the inbred circulars, so that the possibility of a single pair of factors being involved here is eliminated. Multiple factors are perhaps concerned, although it is not entirely clear how the results observed could be produced by multiple factors.

An examination of the ratios for the different types of progeny, in table 1, shows perhaps sufficient agreement in successive conjugations to indicate a constancy in arrangement and behavior of the genetic factors that determine form. The extreme ratios here for any one type of characteristic, in different conjugations, do not differ by a greater amount than approximately 10 percent. Considering the small number of lines on which each ratio is based, and the extent of the inviability both before and after re-

differentiation that removed so many lines from observation, this degree of constancy seems to have some significance.

In section f, above, it was shown that in most (though not all) cases, the descendants of the two members of a pair of conjugants have the same form. In terms of genetic factors, they inherit like sets of determiners for this characteristic. This possibly indicates something about the nature of the segregation of paired factors in the parents. But before examining the data in this connection, it will be necessary first to consider the known cytological facts.

According to the work of TURNER (1930) the maturation processes of *Euplotes patella* are somewhat different from those of most other protozoa in which they have been studied. In typical maturation in protozoa generally (JENNINGS 1929, pp. 131–135), the micronucleus divides twice, forming four, and during these two divisions reduction occurs. Three of the four then disintegrate and the remaining one divides once, forming the two pronuclei, one stationary and one migratory. The unique effect of this process, as compared with meiosis in higher organisms, is that if reduction really occurs only during the first two divisions, the formation of the two pronuclei or gametes from only one of the four reduced products requires that these two gametes be alike genotypically. The consequence of this is that regardless of how diverse the genotypes of two conjugating individuals are, the two ex-conjugants must become exactly similar in genotype as a result of the exchange of pronuclei. It is apparently the occurrence of this in most cases that JENNINGS had in mind as an explanation of biparental inheritance, that is, the tendency for members of pairs to be more alike than two individuals taken at random that had not mated together.

TURNER, however, finds that in Euplotes the maturation phenomena are more complicated. In his account (pp. 212–219), a summary of which is shown diagrammatically in figure 3, it appears that the micronucleus first undergoes a preliminary division and that the first maturation division involves both of these products, forming 4. At the second maturation division, each of the 4 divides and the 8 chromosomes of each become reduced to 4. Of the 8 reduced nuclei, 6 degenerate and the remaining 2 undergo the third maturation division. Of the 4 thus produced, one becomes the stationary pronucleus and one the migratory pronucleus, but it is not possible to tell whether one mother nucleus gives rise to both pronuclei or whether each is derived from a separate mother. So in the one case the two would be alike, in the other they might be different, genotypically.

This uncertainty concerning the immediate origin of the pronuclei prevents making a ready check of the genetic results obtained in the present work by the cytological evidence described by TURNER. It appears, how-

ever, that the genetic evidence may furnish a clue to this doubtful phase of the cytological events.

If the two pronuclei arise from a single mother nucleus (figure 3, first possibility), they must of course have similar genetic factors. However, conjugation between two individuals, each of which has two genotypically similar pronuclei, results in genotypically similar zygote nuclei for the two conjugants, so that by this assumption in all conjugants the two members of a pair should be similar in their inherited characteristics. The genetic

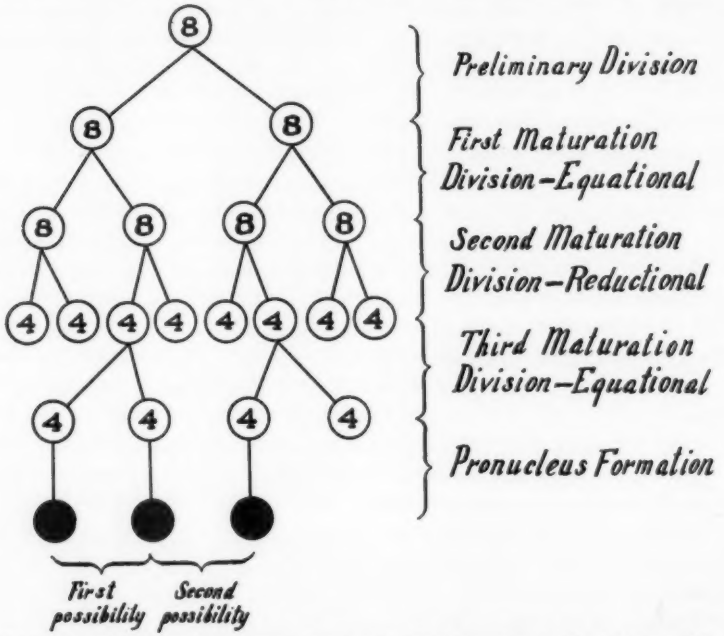

FIGURE 3.—Diagrammatic summary of maturation during conjugation in *Euplotes patella*, based on the account of TURNER (1930). The numerals refer to number of chromosomes.

evidence of the present work, however, shows that in only about 74 percent of the pairs are the two alike in inherited characteristics. The indication is that probably not in every conjugating individual are the two pronuclei formed from the same mother nucleus.

To examine this possibility we may assume that the original micronucleus, before the preliminary division of maturation, is heterozygous for one pair of factors, Aa. Then of the 8 nuclei formed in the reduction division 4 will have the factor A and 4 the factor a. Two of these become the mother nuclei, from one or both of which the gametic pronuclei arise. Since

it is apparently a matter of chance which two of the eight will have this fate, each mother nucleus may have either of the factors, *A* or *a*, the chances for either being 50 percent. If the two pronuclei arise from separate mothers (figure 3, second possibility), in their case also each may have either the factor *A* or the factor *a*, so that the two pronuclei of any conjugant may be *A* and *A*, *A* and *a*, *a* and *A*, or *a* and *a*, the chances for each combination being 25 percent. On the basis of random exchange of pronuclei between two conjugants the expected ratio of like to unlike pairs may be readily calculated and turns out to be 37.5 percent to 62.5 percent. The ratio actually obtained, 74 percent to 26 percent, does not agree with this and so indicates that the two pronuclei do not in every case form from separate mother nuclei.

The genetic evidence, therefore, seems to show that the gametic pronuclei arise sometimes from one mother nucleus, sometimes from two. If in 50 percent of the conjugants they arise from one mother, and in 50 percent from two, the expected ratio of like to unlike pairs is 69 percent to 31 percent which agrees fairly closely with the ratio obtained.

The facts concerning viability cannot all be readily interpreted in terms of Mendelian factors. Low viability may perhaps be due to recessives that are lethal when homozygous. But complete inviability cannot be explained in this way. The significance of this latter phenomenon is in fact quite obscure, since no satisfactory hypothesis of any kind has as yet been advanced for it.

The indications that fission rate and viability are in some way related to the form characteristics in inheritance are of interest. The data may be explained by assuming either that the same factor or factors that determine the circular form also influence fission rate and viability adversely, or that these characteristics are determined by other factors which are distributed with those for circular, and so illustrate linkage of the familiar Mendelian type. The extremely tentative nature of these suggestions, however, warrants no more for the present than their mere statement.

It is perhaps best not to attempt to carry the analysis of the present experimental facts farther than this. The foregoing discussion has been concerned primarily with suggestions for interpretation of these facts, on the assumption that Mendelian factors and Mendelian inheritance are involved in the genetics of Euplotes. It should perhaps be emphasized that this has remained throughout only an assumption, made plausible largely by the fact that inheritance in higher organisms has been successfully worked out with the aid of these concepts. It is, however, by no means clear to what extent they can be applied readily to observations on inheritance in the Protozoa.

There is a great temptation, when confronted with such facts as those

observed in the present work, to take for granted that paired genes, arranged linearly in paired chromosomes, are the determining agents concerned. But genes, of the type known in Drosophila, have not been demonstrated in Protozoa. It still remains possible that some entirely non-Mendelian system may be responsible for many of the observations that have been made in Protozoan genetics.

SUMMARY

1. In the course of conjugation in a clone of normal elliptical Euplotes, the two members of a certain pair gave origin to two lines of circular individuals.

2. These circular individuals when inbred gave origin by conjugation to normal elliptical, circular, and intermediate progeny.

3. These characteristics appeared in four different conjugations of the circulars in approximately the same ratios (averaging about 40 ellipticals to 32 circulars to 28 intermediates).

4. The two lines descended from the two members of the same pair of conjugants may have the same form (EE, CC, or II), or may differ in form (EC, EI, or CI). (E = elliptical, C = circular, I = intermediate.)

5. The proportion of cases in which the two lines from the same pair were alike (about 74 percent of all) was much above what would be produced by random pairing of the different lines resulting from conjugation.

6. The viability after conjugation was low, varying from about 65 percent to 0. This low viability ran through three successive conjugations. In one clone (45a) the viability was 0 in two different conjugations, indicating a genetic peculiarity of this clone.

7. Among the clones of different form produced by inbreeding in a circular clone, the normal elliptical clones were considerably higher in both viability and fission rate than the circular and intermediate clones. The two latter differed little in these respects.

8. The relation of the above facts to the known cytological processes and to Mendelian inheritance is discussed.

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A CONTRIBUTION TO AN UNDERSTANDING OF CROSSING OVER WITHIN CHROMOSOME RINGS OF OENOTHERA

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There are two theories to account for linkage phenomena as observed in Oenothera. (1) Linked genes are always found within the same chromosome, that is, linkage is of the conventional or Drosophila type. The number of linkage groups is determined by the number of chromosomes in the genom, and bears no relation to chromosome configuration (SHULL 1928). (2) In addition to linkage of the ordinary type, genes may be distributed among several chromosomes and yet be linked, such linkage being induced by the cohesion of these particular chromosomes to form the circles so characteristic of Oenothera. The number of linkage groups is consequently determined by the number of chromosome groups, and varies from form to form (CLELAND 1923, 1924, 1926).

Neither of these theories is capable, unaided, of explaining all the facts. But while the difficulties in the way of "theory 1" would seem to be insuperable, an hypothesis supplementing "theory 2" has been advanced which seems to account satisfactorily for the few cases of exceptional behavior which constitute the only difficulty in the way of this theory (DARLINGTON 1931, EMERSON 1931). The present paper is devoted to a discussion of this supplementary hypothesis.

Leaving out of account the relation of the segmental interchange hypothesis to the problem of linkage (which hypothesis would definitely exclude "theory 1," if proved [CLELAND 1933]), "theory 1" meets its Waterloo in the phenomenon of linkage alteration described by RENNER (1925). It is impossible on the basis of "theory 1" to explain how two or more genes can be linked in one complex-combination and be independent in another. The painstaking and brilliant researches of RENNER leave no reason to doubt, however, that such alterations in linkage do occur. Furthermore, the investigations of CLELAND and OEHLKERS (1929, 1930), RUDLOFF (1929, 1930), GERHARD (1929) and EMERSON (1930, 1931) indicate clearly that these alterations are related to and accompany alterations in chromosome linkage. Genes which are linked when the chromosomes cohere into a large circle become independent when they come to lie in a hybrid in which the chromosomes are no longer organized into a large circle, but form several independent pairs. The alteration in linkage relations, therefore, and the correlation between linkage relations and chromosome con-

figuration are facts incapable of explanation by "theory 1," and sufficient in themselves to discredit the theory.

On the other hand, there are a few cases of exceptional behavior which seem opposed to "theory 2." According to this theory, the extent to which genes are linked must parallel the extent to which the chromosomes cohere into circles. *There must not be more linkage groups than there are* (not individual chromosomes in the genom, but) *chromosome groups*. While the cyto-genetic work cited in the previous paragraph shows that a close correlation does exist between the extent of genetic linkage and the extent of chromosome cohesion, nevertheless, a few cases are known in which there seem to be one too many linkage groups, and these constitute the difficulty in the way of "theory 2."

The supplementary hypothesis which seems to explain satisfactorily this apparent excess of linkage groups over chromosome groups has been presented by DARLINGTON (1931) and EMERSON (1931) who have suggested the possibility of ascribing the occurrence of segregation within a circle to *ordinary crossing over*. The argument of EMERSON is especially clear and convincing, being based upon actual experimentation with specific genes in *franciscana* and *franciscana sulfurea*.

When the present paper was first begun, it was with the purpose of extending this hypothesis in detail to those cases which have been cited repeatedly as exceptions to the chromosome cohesion theory and which have caused certain investigators to question its validity. These cases concern (1) the apparent existence in *Lamarckiana* (⊙12, 1 pr.) of three linkage groups, as shown by SHULL; and (2) anomolous behavior of a flower size factor, analyzed by RENNER and others. When our manuscript was well under way, however, a valuable contribution by EMERSON and STURTEVANT (1932) appeared in which these matters are discussed at some length, making it unnecessary for us to discuss them further in detail. We have, therefore, eliminated a large portion of our manuscript, but have thought it well to retain and publish certain portions which summarize more completely than has hitherto been done the chief points in regard to the hypothesis, and which add certain points in regard to its application to the situation in *Lamarckiana*.

THE HYPOTHESIS

1. It is assumed that homologous end-segments of chromosomes pair in synapsis, no matter whether they belong to strictly homologous chromosomes or to chromosomes which are otherwise non-homologous. Whether these end-segments extend only a short way from the ends, as DARLINGTON (1931) suggests, or all the way to the spindle attachment, or their lengths vary in different chromosomes and in different complexes is not known,

but for the purpose of our argument the matter is unessential. (The length of homologous segments will determine to what extent pairing in Oenothera is to be called telosynaptic, parasynaptic, or semi-parasynaptic. If only very restricted regions at the ends synapse and the great bulk of the chromosomes remain unpaired in zygotene and pachytene, as DARLINGTON suggests, the chief contention of the early telosynaptists is supported in Oenothera, for the great bulk of the pachytene spireme would then be *univalent*. If, on the other hand, the homologous segments are long and the greater portion of the length of each chromosome enters into synaptic union with homologous portions of other chromosomes the situation could then be called without hesitation parasynaptic.)

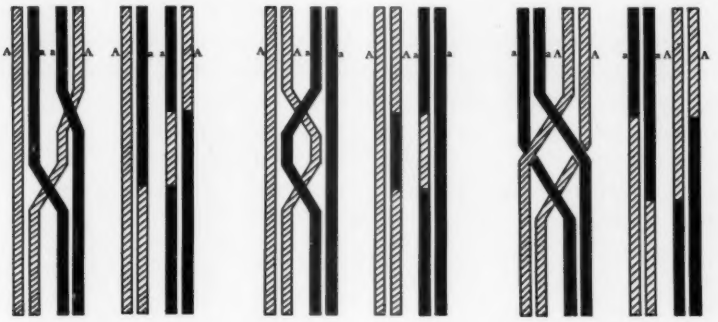
2. To the extent to which pairing takes place, to that extent one might expect crossing over to occur. We may assume that crossing over may occur at any point along the paired segments and that it is of the same type as that found in paired chromosomes.

3. Crossing over would in no way affect chromosome configuration. It would, however, offer a mechanism whereby *complexes could exchange genes* and thus alter linkage relations.

4. The frequency with which a given gene in a circle chromosome becomes transferred to the opposite complex is dependent, not upon the distance from any other gene or group of genes as is true of paired chromosomes, but, as shown below, *upon its distance from the innermost (most proximal) point at which the segment in which it is found is paired with its homologue*. As will be seen in the accompanying diagram (figure 4), each end segment has a distal end which, in a sense, is free and a proximal end which is fixed by being attached to another and non-homologous segment. A gene located in such an end segment will be exchanged to the opposite complex by any single crossover occurring at any point proximal to it. (Assuming that crossing over occurs at the four strand stage, such a crossover will of course affect only two of the four strands.) The gene will, however, remain a part of its original complex if such crossovers as occur are distal to it. Clearly, therefore, genes which are more distally placed will stand a greater chance of being transferred to the opposite complex than will those placed nearer the proximal end of the chromosome segment. It follows that *the frequency with which a given gene is transferred to the opposite complex through crossing over will depend upon its distance from the innermost point at which the segment in which it is found is paired with its homologue.*

The occurrence of double crossing over will probably not affect the total frequency with which a given gene shows transfer from one complex to another. Double crossovers of three types may occur proximally to a given gene. In the first type, three of the four strands are involved in the two

crossovers (figure 1). The result, as far as the relation of the genes *Aa* to the complexes is concerned, is the same as when a single crossover occurs proximally to those genes, that is, in two of the strands the genes (*A* or *a*) are still associated with their original complex, but in the other two they are associated with the opposite complex. In the second type, the same two strands are involved in both crossovers. The two crossovers cancel each other, so far as affecting the relation of *Aa* to the complexes is concerned, so that genes *Aa* undergo no transfer (figure 2). In the third type, different pairs of strands are concerned in crossovers at different levels, with the result that all four strands become crossover strands so far as *Aa* are concerned, that is, *A* or *a* has become associated with the opposite complex in each strand (figure 3).



FIGURES 1–3.—Three types of double crossing over which may occur proximally to a given gene in a circle of chromosomes. Solid bars represent strands belonging to one "Renner complex," hatched bars those belonging to the opposing complex. Explanation in text.

If these three types of double crossing over occur with about the same frequency, it is probable that types 2 and 3 will neutralize each other in their effect on the relation of *Aa* to the complexes, giving a combined effect equal to the average action of single crossovers or of crossovers of type 1. We may then, for all practical purposes, speak as though only single crossovers occur proximally to a given gene, involving two of the four strands.

5. The *detection* of the transfer of a given gene to the opposite complex and the determination of the frequency of such an occurrence are usually complicated by the presence of balanced lethals in the circle. Owing to their presence, recessive genes are prevented from expressing themselves *except* in the case of a crossover. It is the percentage with which *a recessive type appears after selfing*, therefore, that makes it possible to determine the percentage in which the gene responsible for this type is transferred from one complex to the other.

Ability to use the backcross of the heterozygote to the recessive in determining crossover percentage will depend upon the forms used. When the heterozygote is an isogamous form like *Lamarckiana* and the recessive is the same form, the backcross cannot be used, for the impossibility of the survival of anything but complex-heterozygotes from this cross because of the lethals means that a 1:1 ratio will result, irrespective of the percentage in which crossing over occurs (leaving out of consideration such modifying influences as pollen and gone competition). Thus, a 1:1 ratio in backcross is actually obtained in the cases of genes which show such widely divergent ratios after self-pollination as 10:1 (*sulfurea*) and 3:1 (*brevistylis*) (SHULL 1926). In the case of a heterogamous form, however, the backcross can theoretically be used to test crossover percentage.

The difference in this respect between isogamous and heterogamous forms may be made clearer by the following examples in which α and β are gametes and G and g are genes carried by them. Crossover gametes and progeny are enclosed in brackets.

ISOGAMOUS SPECIES

Gametes of heterozygote		*Gametes of homozygote*		*Resultant progeny*
αG	×	βg	=	1:1 $\alpha\beta Gg$
βg	×	αg	=	1:1 $\alpha\beta gg$
(αg)	×	βg	=	1:1 $(\alpha\beta gg)$
(βG)	×	αg	=	1:1 $(\alpha\beta Gg)$

(Theoretical ratio of $\alpha\beta Gg$ to $\alpha\beta gg$ in progeny = 1:1)

HETEROGAMOUS SPECIES

Eggs of heterozygote		*Sperms of homozygote*		*Resultant progeny*
αG	×	βg	=	$\alpha\beta Gg$
(αg)	×	βg	=	$(\alpha\beta gg)$

(Theoretical ratio of $\alpha\beta Gg$ to $\alpha\beta gg$ in progeny = ratio of αG eggs to αg eggs)

In the following table are given the theoretical percentages in which recessive types may be expected to appear as a result of selfing in forms

TABLE 1

PERCENTAGE OF SPOROCYTES IN WHICH CROSSING OVER OCCURS PROXIMAL TO *Aa*	RATIO OF *A* TO *a* AFTER SELFING
10	20.0*A*:1*a*
20	10.10*A*:1*a*
40	5.25*A*:1*a*
50	4.33*A*:1*a*
60	3.76*A*:1*a*
80	3.17*A*:1*a*
100	3.00*A*:1*a*

with balanced lethals, following certain specific crossover percentages. (It is assumed that crossing over occurs at the four strand stage. If it takes place at the two strand stage, only one-half the amount of crossing over will be necessary to produce a given percentage of segregation after selfing.)

From this table it is evident that the occurrence of crossing over proximally to a given gene in 60 percent or more of the cases will result theoretically, after selfing, in an approximation to the 3:1 ratio in the next generation; and even as low as 40 percent will give a ratio that might be taken as 3:1 in small pedigrees (leaving out of consideration such modifying influences as pollen and gone competition).

It may be thought that 40 percent is a high crossover figure, but it must be reemphasized that we are not dealing with the percentage of crossing over between two genes, but rather with the percentage of cases in which *crossing over occurs at any point proximal to a given gene.* It therefore makes no difference at what point the crossing over between segments occurs, so long as it is proximal to the gene. It is clear that, in the case of a gene at the extreme distal end of a segment, crossing over will result in a transfer of this gene *every time* it (crossing over) occurs in this segment.

While we do not know how long the terminal segments are in any of the chromosomes of Oenothera, it is quite probable that they are long enough to allow for at least one crossover in 60 percent of the cases, certainly in some of the segments. Indeed, if DARLINGTON'S crossing over hypothesis be correct, according to which every chiasma represents an antecedent crossover, the average number of crossovers per segment must be somewhat in excess of one (DARLINGTON 1931). The chances are good, at any rate, that terminally placed genes will show, after selfing, a percentage of transfer which will result in an apparent independence of these genes from their complexes. Genes not terminally placed, but lying farther back from the end, will show a corresponding reduction in the percentage of transfer and will segregate out less frequently.

Application of this hypothesis to the situation in O. LAMARCKIANA

SHULL has shown the apparent presence of three independent gene groups in *Oe. Lamarckiana* as follows:

GROUP 1		GROUP 2
sulfurea (*s*) = sulfur flowers		*brevistylis* (*br*) = short style
augustifolia, *stenophylla* = narrow-leaved forms		
		GROUP 3
nanella (*n*) = dwarf stature		*vetaurea* (*v*) = old-gold flowers
pervirens (*p*) = all green	probably	*supplena* (*sp*) = double flowers
Rubricalyx (*Rh*) = red hypanth.	multiple	*bullata* (*bu*) = crinkly leaves
green hypanthium (R^c)	alleles	*Red-vein* (*Rv*) = red midribs of leaves
funifolia (*f*) = revolute leaves		

Rubrifolia (*Rf*) = red on leaves
zygote lethal 1_1
zygote lethal 1_2
(possibly gamete lethals)

acutifolia (*af*) = acute, nearly uncrinkled leaves

The material showing these linkage relations has had, in some cases at least, a circle of twelve and one pair, and probably has possessed this configuration in most of the cases. If the pair be made to account for one of the linkage groups, the remaining two groups are necessarily placed within the circle of twelve chromosomes. This raises the question as to whether it is possible for the circle to be the basis for two linkage groups and, if so, which groups are to be assigned to the circle and which to the pair.

In the first place, it is logical to assume that group 1 is associated with the circle of twelve since it is the largest group. With respect to the other two groups, EMERSON and STURTEVANT (1932) have clearly shown that it is group 3 which belongs to the pair and that *brevistylis*, the sole member of group 2, belongs, in reality, in the circle along with the genes of group 1.

The genes which are located in the circle of chromosomes in *Lamarckiana* are probably situated at different loci along their respective segments. It is extremely improbable that all known genes belonging to the circle lie either so close to the proximal ends of their segments that they never segregate, or so close to the distal ends that they seem to be independent of the complexes. It is reasonable to suppose, therefore, that the segregation frequencies of circle genes which have come to the attention of the Oenothera workers will not be the same. Unfortunately, the list of known genes governing visible characters is not large and crossing over data are scanty. Nevertheless, the following data in regard to segregation frequencies of these genes indicate that they lie at varying distances from the distal ends of their segments.

Rubrifolia, which appeared as a dominant mutation, has given, by selfing, progenies consisting entirely of *Rubrifolia*. These plants, however, have been shown to be heterozygous, since backcrossing to the recessive type gave segregating progenies (new data). A single possible case of crossing over has been found involving this gene. In a family of 51 individuals from the selfing of *Rubrifolia* one green rosette appeared (SHULL, private correspondence). The gene *Rf* no doubt lies very close to the proximal end of the segment.

Nanella, a recessive gene, segregates out from *Lamarckiana* in about 0.5 percent of the population, a phenomenon probably due to crossing over. Its locus is apparently quite close to the innermost point at which its segment is paired with its homologue.

Pervirens appeared originally as one plant in a family of 79 (SHULL 1921). From data of SHULL (1932), it appears that this factor segregates out very rarely after selfing a plant heterozygous for it. A summarization

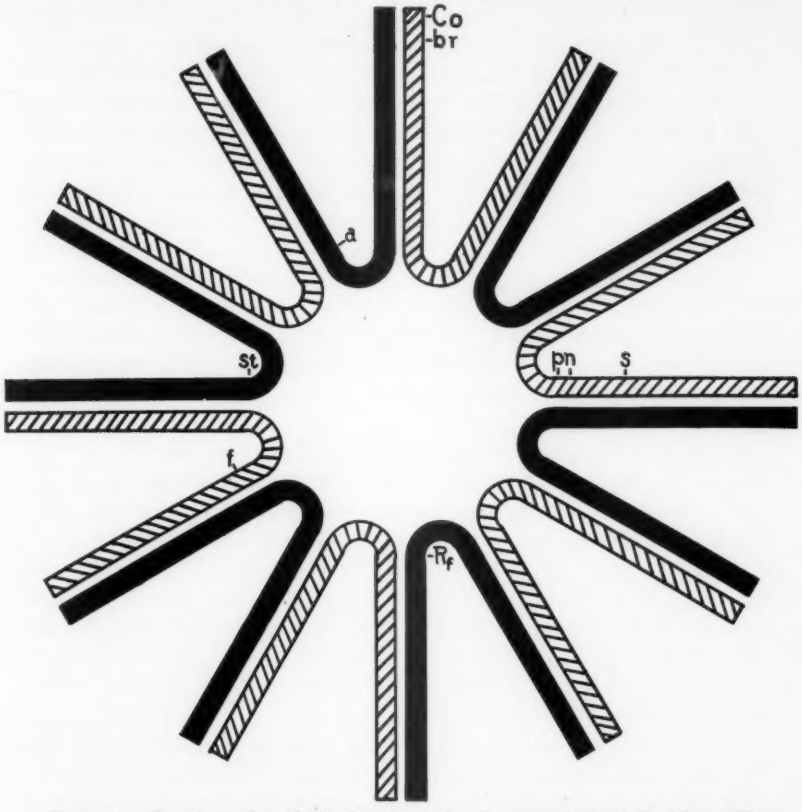


FIGURE 4.—The relative (not absolute) positions of various genes in the circle of *Lamarckiana* based upon crossover ratios. Data of EMERSON (1931) indicate that *p*, *n*, and *s* are in the same chromosome, possibly in the same segment. Data of EMERSON and STURTEVANT (1932) indicate that *Co* and *br* are also in a single chromosome, possibly in the same segment. Genes are placed in separate chromosomes in the absence of data to the contrary. The relative positions of *Co* and *br* are unknown. It is also unknown to which complex most of the genes belong. Black and hatched chromosomes belong to opposing complexes.

of the data in tables 1, 3, and 4 of SHULL (1932) shows a total of but 9 *pervirens* crossovers in a total of 4073 plants derived from selfing plants heterozygous for *pervirens*. This suggests that the *pervirens* gene lies so close to the inner end of its segment that crossing over occurs very rarely at loci proximal to it.

There is strong reason to believe that *Rubricalyx* and red cone are alleles of *pervirens*. Either that, or they are very closely linked. One would expect them all to show, therefore, about the same percentage of crossing over. In the absence of data in this regard, no further statement can be made.

Stenophylla arose in 1925 from the trisomic mutant *Oe. liquida* (DE VRIES 1929). It has never arisen directly from *Lamarckiana* itself, either in the experience of DE VRIES or in that of SHULL (private correspondence) who received the strain from DE VRIES. It is evident that the gene for this character resides close to the proximal end of its segment. It is even possible that the segment in which it lies is longer than its homologue in *Lamarckiana* and hence that crossing over of this gene is impossible in straight *Lamarckiana*.

Angustifolia arises almost every year from *Lamarckiana* in the experience of DE VRIES (1929). SHULL, whose material came from DE VRIES, finds also that this form appears occasionally in his *Lamarckiana* cultures (private correspondence). No ratios have been given, but it is evident that this gene lies far enough out on its segment to be crossed over with fair frequency to the opposite complex.

Funifolia. This character appeared originally in five plants in a family of 100 after selfing a plant which was presumably heterozygous for this gene (SHULL 1921). It has not subsequently appeared, inasmuch as the line from which it sprang has not been carried on. The appearance, however, of several plants bearing the new recessive character suggests that they arose by crossing over, and that the *f* locus is far enough removed from the most proximal point of pairing of its segment to make crossing over proximal to this gene possible in something like 10 percent of the sporocytes, thus resulting in segregation of the recessive type in approximately 5 percent of the population.

Sulfurea segregates out in selfed line in a ratio of about 10:1, as shown by SHULL (1926). He obtained a total of 943 non-*sulfurea* to 94 *sulfurea*. In backcrosses, however, the 1:1 ratio was approximated, there having been produced a total of 514 non-*sulfurea* to 508 *sulfurea*. This would indicate that *s* lies at such a distance from the inner end of its segment that crossing over occurs proximally to it in about 20 percent of the sporocytes, resulting in a 10 percent segregation of the recessive condition.

Brevistylis. A summary of SHULL'S data (1926, 1928) shows *brevistylis* to have appeared, upon the selfing of heterozygotes, in 47 plants out of 635, or in the ratio of 3.32:1. The locus of *br* is close enough to the distal end of its segment so that crossing over occurs proximally to it in from 75 percent to 100 percent of the sporocytes, resulting in an approximation to a 3:1 ratio.

To these genes must undoubtedly be added, as has been shown by EMERSON and STURTEVANT (1932), another factor, one for flower size, which has an extremely distal position in its segment.

These data are, on the whole, meagre. Nevertheless, they furnish some evidence that the loci of the various genes are differently placed in relation to the ends of their respective segments, some lying close to the proximal ends, others in intermediate positions, still others close to the distal ends. The relative (not absolute) positions of these genes are schematically represented in the accompanying diagram (figure 4), it being tentatively assumed where there is absence of evidence to the contrary that they lie in different segments. On the whole, it seems reasonable to postulate that the varying degrees with which recessive genes belonging to group 1 become detached from their lethals and segregate out is due to their being located at varying distances from the proximal ends of their segments. The genes *br* (*brevistylis*) and *Co* (flower size) merely represent, therefore, the extreme in a series of genes in group 1 which show more or less segregation from their lethals.

CONCLUSION AND SUMMARY

In conclusion, we may say that the suggestion made independently by DARLINGTON and EMERSON that crossing over might occur between the paired strands of the end-segments of Oenothera chromosomes, transferring genes situated distally to the crossover to the opposite complex, may be applied to those cases which have been cited specifically in opposition to the chromosome cohesion theory. If this hypothesis be true, cause for controversy among proponents of opposing theories of linkage in Oenothera disappears. It is evident that chromosome cohesion results in the linkage of genes situated in different chromosomes. It is probable, however, that one or more genes lying close to the distal ends of their respective segments are transferred by crossing over to the opposing complex with frequencies sufficient to render them apparently independent of their complexes.

Unfortunately, the number of known genes is too small to make feasible genetical tests of this hypothesis. Such tests must await the accumulation of a larger number of genes. There can be little doubt, however, that, as matters stand, the chromosome cohesion theory supplemented by the crossing over hypothesis constitutes by far the most satisfactory explanation of genetic behavior in Oenothera, and the only explanation that does not go counter to known facts.

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INHERITANCE OF RESISTANCE TO BUNT, TILLETIA TRITICI, IN SHERMAN AND ORO WHEAT HYBRIDS

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INTRODUCTION

In previous publications, the inheritance of resistance to bunt, *Tilletia tritici* (Bjerk) Wint., in hybrids with eight resistant varieties of wheat, has been reported. The resistance of Martin (BRIGGS 1926), White Odessa (BRIGGS 1930b), Banner Berkeley (BRIGGS 1931), and Odessa (BRIGGS 1932b) wheat varieties has been shown to result from the same dominant factor in each case. This factor has been designated as the Martin factor (*M*). Hussar wheat (BRIGGS 1926, 1930a) depends on two factors for its resistance, the Martin and Hussar (*H*) factors. This second factor is not completely dominant, as bunt occurs on a part of the heterozygous plants. Selections 1418 and 1403 each have only the Hussar factor. Turkey 1558 and Turkey 3055 (BRIGGS 1932a) each have a single factor for resistance to this disease which has been designated as the Turkey factor (*T*). This factor is similar to the Hussar factor in effect.

Data are presented here to show the number of factors for resistance to bunt in Sherman and Oro wheats. The crosses necessary for identifying these factors are available.

METHODS AND MATERIALS

The parental varieties and hybrid populations were grown in the field at the University Farm, Davis, California. The method of handling and the source of inoculum have been described in a number of the publications referred to above. A single collection of bunt has been propagated and used continuously since 1917.

TABLE 1

Annual percentages of bunt infection at Davis, California in the parent wheat varieties during the years indicated.

VARIETY	PERCENTAGE OF BUNTED PLANTS				
	1929	1930	1931	1932	AVERAGE
Oro	0	0	0	0.1	.03
Sherman	0	0	0	0.1	.03
Martin	0	0	0	0	0
Turkey 3055	0.1	2.0	1.2	1.8	1.3
Baart			47.2	85.5	66.3
White Federation	78.6	59.3	43.0	73.2	63.5

Seed of Sherman and Oro were supplied in 1928 by Dr. K. S. QUISENBERRY, Division of Cereal Crops and Diseases, UNITED STATES DEPARTMENT OF AGRICULTURE. These varieties were known by Dr. QUISENBERRY to be resistant to bunt. The record of these varieties at Davis may be seen in table 1.

Duplicate rod rows of Sherman and Oro were grown in 1929 and 1930. Four rod rows of each were grown in 1931 and 20 rod rows in 1932. One plant of Sherman and two of Oro became infected in 1932. Compared with these resistant varieties, Baart and White Federation may be considered completely susceptible.

Sherman and Oro each were crossed with Baart to determine the number of bunt-resistant factors in each of these two resistant varieties. Crosses also were made with Martin and Turkey 3055 to test for the presence of the Martin and Turkey factor respectively. The cross with Selection 1403, which is the tester for the Hussar factor, was not obtained because of the difference in heading dates. As will be seen later, this cross was not necessary for identifying the resistant factors in Oro and Sherman wheats.

TABLE 2

Percentage of bunted plants in the parents and F_2 of the crosses named. Grown at Davis, California.

PARENT OR CROSS	YEAR GROWN	PLANTS GROWN NUMBER	PLANTS BUNTED	
			NUMBER	PERCENT
Sherman	1931	185	0	0.0
	1932	1265	1	0.1
Oro	1931	176	0	0.0
	1932	1305	2	0.2
Turkey 3055	1931	145	0	0.0
	1932	622	9	1.5
Martin	1931	86	0	0.0
	1932	434	0	0.0
Baart	1931	362	170	47.0
	1932	2370	2061	87.0
Sherman×Baart	1931	641	92	14.4
	1932	623	168	27.0
Sherman×Turkey 3055	1931	483	14	3.2
	1932	684	48	7.0
Martin×Sherman	1931	478	0	0.0
	1932	871	0	0.0
Oro×Baart	1931	585	103	17.6
	1932	1264	412	32.3
Oro×Turkey 3055	1931	499	3	0.6
	1932	679	7	1.0
Martin×Oro	1931	427	8	1.9
	1932	894	24	2.7

EXPERIMENTAL RESULTS

F_1 seeds were not inoculated because of the small number available. Where F_2 plants were being grown as a source of seed to be inoculated in F_3, they were kept free from bunt. By doing this, there was no elimination of susceptible progeny by bunt in F_2.

F_2 data do not permit a satisfactory Mendelian analysis because some susceptible plants usually escape infection, and resistant and heterozygous plants may be infected occasionally. An F_2 population was inoculated the year previous to growing the F_3 and another population the same year as the F_3. These data are of value because they give some indication of the number of factors by which the resistant and susceptible varieties differ. Also they indicate the percentage of bunt to expect in F_3 rows of the same genotype. Therefore, the F_2 data are given in table 2.

In 1931, the percentages of bunt in the parent varieties, as well as in the F_2 populations were only about half those secured in 1932. In fact, the infection in 1931 was the lowest obtained during the 13 years that records have been available here. That a number of susceptible F_2 plants escaped infection in 1931 may be seen from the fact that the F_2 of Sherman × Baart had 14.4 percent of bunted plants that year and 27.0 percent in 1932. Similar results were secured with the other crosses. The classification of F_2 plants on the basis of bunt obtained in F_3 rows is much more satisfactory. F_3 rows contained from 30 to 70 plants, usually about 50. These data may be seen in table 3.

The rows in the 0–5 percent class for bunt infection were subdivided into those without bunt and those with 1–5 percent because the former group always is of interest.

The hybrids with Sherman may be considered first. The distribution of rows in the Sherman × Baart cross is shown in figure 1. This curve resembles the Martin curves previously published (BRIGGS 1926, 1930b, 1931, 1932b). The number of rows under the three modes agrees satisfactorily with the 1:2:1 ratio. Accepting 7.5 and 52.5 as minima, there are 54.5 resistant, 146.5 heterozygous and 70 susceptible rows where 67.75, 135.5, and 67.75 were the numbers expected. This gives a value of P greater than 0.1. The heterozygous F_3 rows, which are of the same genotype as the F_2 population, averaged 24.2 percent of bunt compared with 27.0 percent for the F_2 grown in the same nursery. The susceptible rows had 82.2 percent of diseased plants under the same conditions that Baart produced 87.0 percent. The data then indicate that Sherman differs from Baart in one major factor for resistance to bunt. This factor is similar to the Martin factor in effect.

The identity of the factor in Sherman is established by the cross with

TABLE 3

Distribution of parent and F_2 rows of the crosses named into 5 percent classes for bunt infection, when grown at Davis, California, 1932.

PARENT OR CROSS	DISTRIBUTION OF ROWS HAVING A BUNT INFECTION (PERCENT) OF																					TOTAL NUMBER ROWS
	0–5		5–	10–	15–	20–	25–	30–	35–	40–	45–	50–	55–	60–	65–	70–	75–	80–	85–	90–	95–	
	0:	1–5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	
Sherman	17	1																				18
Oro	16	2																				18
Turkey 3055	4	3	1																			8
Martin	6																					6
Baart																	3	9	13	9	1	35
Sherman×Baart	28	26	1	4	27	64	28	17	4	0	2	0	1	2	3	7	12	17	15	10	3	271
Sherman×Turkey 3055	65	24	20	6	7	8	3	2	1	0	0	1	1	0	1	1						140
Martin×Sherman	140																					140
Oro×Baart	18	45	18	7	17	20	30	27	19	21	8	5	9	9	7	13	11	7	3	2	1	297
Oro×Turkey 3055	71	57	9																			137
Martin×Oro	50	23	11	7	3	2	3	0	0	2	0	1	1	0	1							104

Martin. The fact that these hybrids did not segregate for bunt resistance shows that Sherman is identical with Martin in its resistance to this fungus.

The cross of Sherman with Turkey 3055 may be considered briefly even though it did not happen to be necessary for establishing the identity of the resistant factor in Sherman. It was made for that purpose at the same time the other crosses were made. As would be expected in the light of the above data, this cross gave a segregation typical of those obtained previously with two factors (BRIGGS 1926, 1930a). In such a cross, the susceptible rows are the only ones that can be recognized readily. There were 4

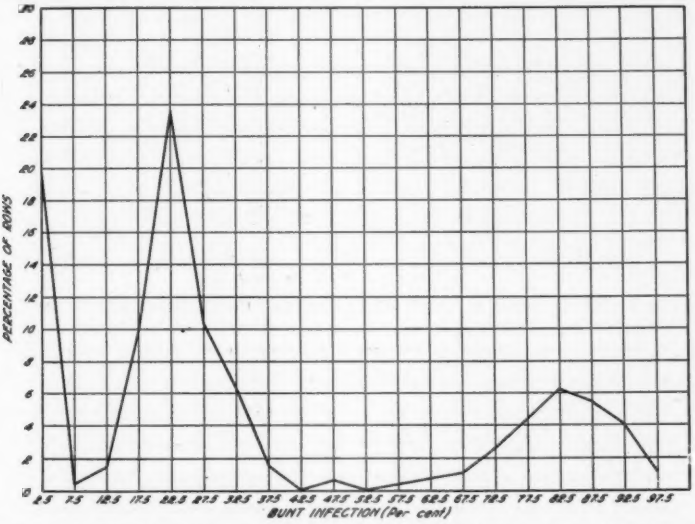

FIGURE 1.—Distribution of F_3 rows of Sherman×Baart into 5 percent classes for bunt infection.

susceptible rows where 8.8 were expected, thus giving the value for P between 0.1 and 0.2.

Considering now the crosses with Oro it is interesting to compare these with the Sherman crosses. As will be seen presently, these two varieties depend on different genetic factors for their resistance to bunt. The Oro× Baart hybrids gave a monohybrid ratio as shown by figure 2. There were 84.5 resistant, 148 heterozygous, and 64 susceptible F_3 rows where 74.25, 148.5 and 74.25 respectively were the numbers expected, giving P a value greater than 0.2. The F_2 of Oro×Baart had an average of 32.3 percent of bunt compared with 31.4 percent for the heterozygous F_3 rows. This is 7.2 percent more than found on the heterozygous rows of Sherman× Baart, indicating that some heterozygous plants become infected.

The susceptible rows of Oro×Baart had an average of 71.4 percent of bunt which is lower than produced by Baart. This does not appear to be

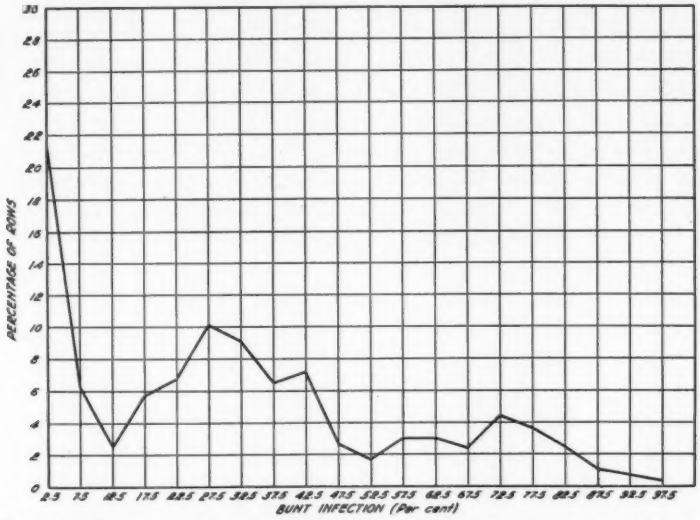

FIGURE 2.—Distribution of F_3 rows of Oro×Baart into 5 percent classes for bunt infection.

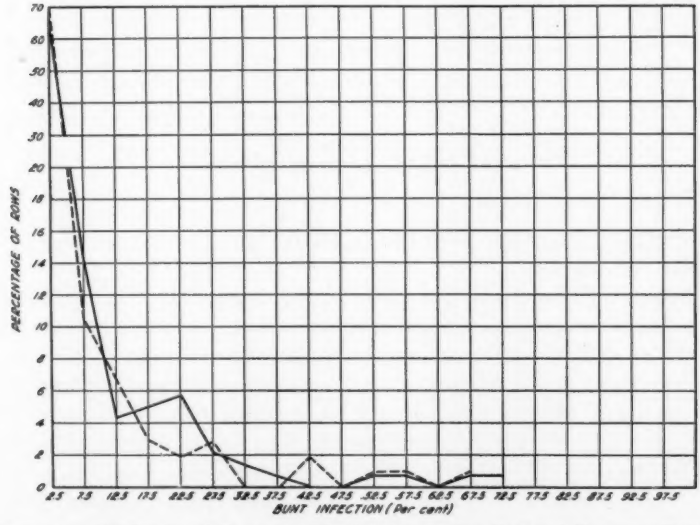

FIGURE 3.—Distribution of F_3 rows of Martin×Oro (broken line) and Sherman×Turkey 3055 (solid line) into 5 percent classes for bunt infection.

due to place effect in the nursery because the rows of Baart grown within the same area had percentages of bunt about average for that variety. The reasons for the lower percentage of bunt for the F_3 rows are not known. It may be due in part at least to modifying factors.

The Oro×Baart curve is distinctly different from the Sherman×Baart curve which is typical for the Martin factor. It is similar, however, to the curve resulting from the Hussar factor (BRIGGS 1930a) and the Turkey factor (BRIGGS 1932a). That the Turkey factor, but not the Hussar factor, is responsible for the resistance of Oro is shown by the Turkey 3055×Oro cross. No susceptible rows were found in a population of 137 rows. This cross averaged 1.3 percent of bunt which is slightly more than the average of the parents.

Oro was crossed with Martin at the time the other crosses were made. This was not a critical cross for identifying the bunt resistant factor in Oro. However, it is interesting to compare it with the Sherman×Turkey 3055 cross because the same two bunt resistant factors are involved in each cross but contributed in each case by different varieties. This comparison may best be made by referring to figure 3. These curves are very similar considering the relatively small number of rows grown from each cross.

DISCUSSION AND SUMMARY

Data were presented to show that Sherman and Oro differ from the susceptible variety, Baart, in one major factor for resistance to bunt. The factor in Sherman was identified as the Martin factor and the one in Oro as the Turkey factor. This makes ten varieties that have been studied. The genetic constitution of these as regards bunt resistance may be seen in table 4.

TABLE 4

The genetic constitution of ten bunt resistant varieties of wheat as determined by the author.

VARIETY	BUNT RESISTANT FACTORS
Martin	*MM hh tt*
White Odessa	" " "
Banner Berkeley	" " "
Odessa	" " "
Sherman	" " "
Hussar	*MM HH tt*
Selection 1418 and 1403	*mm HH tt*
Turkey 1558	*mm hh TT*
Turkey 3055	" " "
Oro	" " "

The first five varieties depend on the Martin factor, *M*, for their resistance to bunt. Hussar has the Hussar factor, *H*, in addition to the *M* factor. Selections 1418 and 1403 which are from Hussar×Hard Federation only

have the *H* factor. The last three varieties, Turkey 1558, Turkey 3055, and Oro each have the Turkey factor, *T*, only.

It is entirely possible that in some of these varieties there are other factors for resistance to bunt which are not apparent in the presence of the collection of bunt used in these experiments. If the *M* factor discovered in Martin, White Odessa, Banner Berkeley, Odessa, and Sherman is the only factor for resistance to bunt in these varieties, they should react the same to each collection of bunt. If some other resistant factor is present in one or more, but not in all five varieties, a differential reaction should be obtained with some collections. Furthermore, Hussar should be resistant to all collections to which Martin is resistant but in addition should be resistant to some which attack Martin, because of the presence of *H*. Finally, if the above analysis is correct, only three differential hosts are available in the above list of varieties. Other resistant factors undoubtedly exist in other varieties. Recently, CHURCHWARD (1931 1932) has published data to show that Florence differs from susceptible varieties in a single recessive factor. This factor must be different then from any of the three listed above.

The predictions made above may be tested out in so far as data are available. REED (1928), GAINES (1928), and BRESSMAN (1931) have studied the reaction of some of the above varieties to a number of collections of bunt. In general, their results agree with the genetic interpretation given in table 4. Considering REED's results first, comparisons are available for Martin, Odessa, and Hussar. He used two strains of Turkey which were different from each other. Since their relation to the above strains of Turkey is not known, they will not be considered. There is one outstanding difference in the infection of Martin and Odessa by one collection of bunt. The collection of *Tilletia tritici* from West Seneca, New York, produced 54 percent of bunt on Odessa but only an average of 7 percent on Martin. This same collection produced 8 percent on Hussar 527, and 23 percent on Hussar 814. This suggests the presence of a factor for resistance to bunt in Martin which is not present in Odessa or perhaps Hussar wheats. In no other case is Martin resistant to a collection which readily infects Odessa or Hussar. As might be expected, he found a case in which Hussar was resistant but Martin and Odessa susceptible. Hussar's resistance here may be attributed to the *H* factor.

GAINES (1928) reported one case where 32 percent of White Odessa plants were infected but only 1 percent of Martin and 1 percent of Hussar plants showed the disease. Otherwise his data conform to those of REED.

BRESSMAN (1931) inoculated a number of resistant varieties with various collections of bunt. He states: "White Odessa, Martin, Banner Berkeley, and Regal gave results similar to those obtained with Albit."

Regal and Albit have not been studied by the author. However, Albit may be assumed to have only the *M* factor for resistance because it is a selection from Hybrid 128 × White Odessa made by GAINES at WASHINGTON STATE COLLEGE (CLARK, PARKER and WALDRON 1927). Hybrid 128 is very susceptible and probably contributed nothing to the resistance of Albit. A few cases were found where some one of these varieties appeared to be susceptible while the others were resistant. For example, collection 7c produced 26 percent of bunt on White Odessa in 1928 and only 3 percent on Martin and 1 percent on Banner Berkeley. The following year at Corvallis these varieties had 0, 3, and 6 percent respectively, indicating that White Odessa probably is resistant to this collection. Other similar cases were encountered.

Results at Corvallis in 1929 made possible a comparison of Albit and Hussar in their reaction to all 94 collections of bunt. In a number of cases, Hussar is resistant to collections but Albit is susceptible. Hussar's resistance again may be attributed to the *H* factor. In every instance, with the possible exception of collection 4, if Hussar is susceptible Albit also is susceptible. BRESSMAN uses the results with collection 4 at Corvallis 1929 as a type for his physiologic form II, presumably because Hussar produced 20 percent of bunt, while Albit had 6 percent, Ridit 4 percent, and Oro 3 percent. However, the year before, Albit had 12.5 percent of bunt and Hussar had 1.5 percent.

If the assumption that Albit has only the Martin factor for resistance is correct, then three of the four varieties finally chosen by BRESSMAN as differential hosts each have a different genetic constitution as seen in table 4. Albit, Hussar, Oro, and Ridit were the varieties used by him.

Ridit is different from the other three varieties in its reaction to these collections of bunt. The genetic constitution of Ridit is not known at present, but something is known about the genetics of its parents. This variety was selected by GAINES at WASHINGTON STATE COLLEGE from a cross of Turkey with Florence (CLARK, LOVE and PARKER, 1926). As pointed out earlier, CHURCHWARD (1931, 1932) believes that the resistance of Florence is due to a single recessive factor. The writer has shown that the resistance of Turkey depends on the *T* factor. Ridit then may be assumed to have either one or both of these factors. In this connection, a comparison of the reaction of Ridit to the various collections of bunt with that of Oro is of interest. Oro is known to have only the *T* factor. Forty-three comparisons are available from BRESSMAN'S data. Oro is resistant to 42 of these collections, and Ridit is also resistant to these same collections. This may be attributed to the *T* factor. However, Oro is susceptible to collection 28, but Ridit is resistant to it. Ridit's resistance to this collection may be

attributed, for the purpose of this discussion, to the factor inherited from Florence.

Recently, SMITH (1932) has differentiated three physiologic races of bunt by the reaction of Martin. Martin was resistant to T1, gave 19 percent of bunted heads with T2, and 71 percent with T3. Furthermore, bunted heads resulting from T2 had very small smut balls. BRESSMAN (1931) reported a few abnormalities with some of his collections. The reaction of Martin to the above three forms of bunt is not readily explained on the basis of a single factor. It is entirely possible that the difference in reaction to T1 and T2 may be due to a modifying factor.

From the above discussion, it is apparent that a genetic analysis of other resistant varieties is desirable. Also more data are needed on the physiologic races of this disease. Pure lines of the fungus are highly desirable in this connection. BRESSMAN (1931) found that some of the collections of bunt received by him were a mixture of the two species of Tilletia. It seems equally certain that some of the collections may have been mixtures of physiologic forms. Improvements in the purity of the inoculum are being made.

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TEMPERATURE EFFECT ON INTERFERENCE AND CROSSING OVER

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A consideration of the data presented in an earlier paper (GRAUBARD 1932) brings out certain facts with regard to the mechanism of crossing over in chromosome II of *Drosophila melanogaster* which may have general significance. In the previous work experiments were performed to study crossing over and interference along the entire length of the normal chromosome II and of the same chromosome with a median inverted section of an estimated length of 25 units. The cross was arranged so as to have eight marked genes along the chromosome covering it from one end to the other. The genes employed were aristaless (3.0) dumpy (13.0) black (48.5) purple (54.5) cinnabar (57.5) vestigial (67.0) Lobe (72.0) and speck (107.0) (MORGAN, BRIDGES and STURTEVANT 1925).

If the data given in tables 8 and 9 of the previous paper are restated so as to show the relative proportions of crossover and non-crossover flies, we get the following:

TABLE 1

Normal chromosome.

CROSSOVERS	14°C	PERCENT	16.5°C	PERCENT	25°C	PERCENT	30°C	PERCENT
0	862	24.5	1417	24.7	1623	30.7	1218	27.0
1	1608	45.7	2629	45.8	2528	47.8	2008	44.5
2	876	24.9	1491	26.0	1043	19.7	1067	23.6
3	157	4.5	190	3.3	85	1.6	201	4.6
4	9	0.25	12	0.2	5	0.1	17	0.4
5	0						2	
6	0						1	
Total	3512		5739		5284		4514	

TABLE 2

Homozygous inversion-bearing chromosome.

CROSSOVERS	14°C	PERCENT	16.5°C	PERCENT	25°C	PERCENT	30°C	PERCENT
0	1094	30.8	1675	31.3	3221	34.2	1319	26.0
1	1686	47.5	2508	46.9	4389	46.7	2170	44.2
2	677	19.0	1053	19.7	1635	17.5	1180	24.1
3	87	2.3	101	1.9	146	1.5	212	4.3
4	4	0.1	5	0.1	7	0.07	14	0.3
5							4	0.1
Total	3548		5342		9398		4899	

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These results indicate that taking the chromosome as a whole the number of non-crossovers is about 25 percent of the total at 30°C, and invariably higher at 25°C. The rest of the flies are distributed among the various types of single and multiple crossovers. In the same paper experiments were also performed on crossing over in only one arm of the second chromosome, a procedure made possible by introducing an inversion in the respective other arm and keeping it heterozygous. Such a condition allows crossing over to occur freely in only one arm. It must be borne in mind that the two arms are joined at the point of spindle fiber attachment to form a V-shaped chromosome. Restating these data (tables 7 and 6) as before, we get the following:

TABLE 3

Heterozygous left inversion. Crossing over takes place in right arm only.

	14.5°C	PERCENT	25°C	PERCENT	30°C	PERCENT
0	2042	55.6	601	53.1	1039	48.7
1	1518	41.5	495	43.8	942	44.2
2	96	2.7	34	3.0	145	6.8
3	1	0.1	1	0.1	4	0.2
Crossovers	1615	44.4	530	46.9	1091	51.3
Total	3657		1131		2130	

TABLE 4

Heterozygous right inversion. Crossing over takes place in left arm only.

	16°C	PERCENT	25°C	PERCENT	30°C	PERCENT
0	2178	54.2	2225	59.9	863	51.6
1	1757	43.7	1437	38.7	725	43.3
2	79	1.9	51	1.4	77	4.6
3					4	0.3
Crossovers	1836	45.8	1488	40.1	806	48.4
Total	4014		3713		1669	

In this case we see that non-crossovers are about twice as frequent as before, giving values of approximately 50 percent at 30°C, and higher figures for the other temperatures. It is therefore apparent that whenever only one arm of this V-shaped chromosome is concerned the chance for at least one crossover is approximately 1/2 at 30°C, but that when the entire chromosome is involved the chance for a crossover is 3/4. At 25°C the probability for getting a crossover in each arm is less than 1/2 and for the entire chromosome the chances are less than 3/4. It should be remembered that the data of DOBZHANSKY (1931) show that the point of spindle fiber attachment is barely to the right of purple and that the two arms are approximately of the same length.

Tables 1 and 2 seem to show that except at 30°C the percentage of non-crossovers is higher for the inversion-bearing chromosome than for the normal.

The data of tables 8 and 9 can be further restated so as to show whether the probability for a crossover or a non-crossover in one arm is equally 1/2 at 30°C with a respective diminution at 25°C when there is free crossing over along the entire chromosome. Under those circumstances we can observe crossing over in one arm while the same process goes on unhindered in the other one as well. This is shown in table 5 where values are also given for the expected and obtained proportions of simultaneous crossovers in both arms.

TABLE 5

Percentage crossovers in each arm and simultaneous crossovers.

	NORMAL CHROMOSOME						
	LEFT ARM	PERCENT	RIGHT ARM	PERCENT	SIMULTANEOUS	PERCENT	EXPECTED
14°C	1713	48.7	1728	49.2	874	24.9	24.0
16.5°C	2846	49.6	2911	50.8	1455	25.3	25.2
25°C	2239	42.3	2424	45.8	1021	19.3	19.40
30°C	2103	46.6	2256	50.0	1060	23.4	23.3
	INVERSION-BEARING CHROMOSOME						
	LEFT ARM	PERCENT	RIGHT ARM	PERCENT	SIMULTANEOUS	PERCENT	EXPECTED
14°C	1757	44.4	1518	42.8	642	18.1	19.0
16.5°C	2354	44.1	2442	45.7	1029	19.2	20.1
25°C	3527	37.5	4173	44.4	1563	16.7	16.7
30°C	2260	46.0	2433	49.5	1127	23.0	22.8

The fact that approximately 50 percent crossovers are obtained in each arm means that at 30°C the probability for getting a non-crossover in each arm is half, and the probability for a crossover also half. Actually this is another way of saying that the map distance of that arm is about 50 units. The probability of getting a non-crossover along the entire chromosome, that is, in both arms, will then be 1/4. The percentage values of tables 1 and 2 show that such is the case. It would also follow from the independence of crossing over in both arms that the probability of getting a simultaneous crossover in both arms should be approximately 1/4 at 30°C and the product of the respective probabilities for each arm at the other temperatures. The last two columns of table 5 give the obtained and expected percentages for simultaneous crossovers. The expected figures are calculated as the product of the actual crossover values obtained for each arm.

It is seen that for no temperatures is there any marked interference. Also at 30°C the obtained values approach 25 percent. The total diminution in crossing over for the inversion-bearing chromosome is again appar-

rent in the percentages of simultaneous crossovers which are lower than normal for all temperatures but 30°C.

A comparison of the total crossover values in the left arm which happens to be normal, given in table 4, with the normal left from table 5, shows that in the former case when there is no crossing over in the right arm the values are 45.8 percent at 16°C, 40.1 percent at 25°C, and 48. percent at 30°C and in the latter when there is free crossing over in the right arm we get 49.6 percent at 16.5°C, 42.3 percent at 25°C and 46.6 percent at 30°C. The same comparison can be made for the right inversion-bearing arm. Table 3 shows that when there is no crossing over in the left arm the values for the right are 44.4 percent at 14°C, 46.9 percent at 25°C and 51.3 percent at 30°C. Table 5 gives 42.8 percent at 14°C, 44.4 percent at 25°C and 49.5 percent at 30°C, when there is crossing over in the left. Taking the two sets together we are justified in saying that the values are not significantly different. Hence it may be concluded that whatever happens in one arm by way of crossing over has no influence on the other.

Cytological evidence (DARLINGTON 1932) indicates that the mechanism of crossing over occurs as shown in figure 1. It can thus be seen that chi-

FIGURE 1.—Diagrammatic representation of crossing over. Only two strands cross at any given point.

asmatization, that is, in DARLINGTON'S sense, the coming together of two strands which have undergone interchange, must not necessarily be followed by genetic crossing over because only two strands chiasmatize at one point. Genetic evidence (ANDERSON 1925, BRIDGES and ANDERSON 1925, REDFIELD 1930) had established that fact independently of cytological observation. Therefore when chiasmatization takes place, the probability of a genetic crossover at that point is 1/2 and that of a non-crossover also 1/2. On this basis we can correlate the crossover ratios shown by the data presented in this paper with DARLINGTON'S mechanism of chiasma formation. It should then follow that at 30°C chiasmatization must occur on the average at least once in each arm of the second chromosome, because the probability for a crossover in that region is 1/2. At 25°C the number of chiasmata in each arm of the chromosome should be less than at 30°C. The number of chiasmata in chromosome II should therefore be between 2 and 2.5 because of the raising of the minimum value by the few triples and quadruples. That figure is in agreement with DARLINGTON'S computation of a mean of over 2 chiasmata in chromosome II. Should this frequency of chiasmata be verified cytologically it would prove

that there is no chiasmatization or crossing over between sister strands. While not proving DARLINGTON'S assumption that chiasmatization occurs only after crossing over has taken place, it would prove that each chiasma results in a genetic crossover whether the crossed strand goes into the egg or not. It is assumed of course, that strands are distributed in a random manner. If DARLINGTON'S hypothesis that chiasmatization always results in a potential genetic crossover is wrong, then this simple correlation of two chiasmata to one genetic crossover should not hold. By the preponderance of chiasmata over genetic crossovers other theories concerning the relation of chiasmatization to crossing over can be tested.

If we next arrange the data of tables 8 and 9 of the previous paper to show the proportions of double crossovers in each arm of the normal and inversion-bearing chromosomes, we observe the following:

TABLE 6

Proportion of double crossovers.

	NORMAL CHROMOSOME						
	LEFT ARM	PERCENT	RIGHT ARM	PERCENT	SIMULTANEOUS	PERCENT	EXPECTED
14°C	141	4.0	190	5.4	8	.22	.21
16.5°C	196	3.4	248	4.3	10	.17	.15
25°C	75	1.4	152	2.8	4	.07	.04
30°C	183	4.0	290	6.4	13	.29	.26
	INVERSION-BEARING CHROMOSOME						
	LEFT ARM	PERCENT	RIGHT ARM	PERCENT	SIMULTANEOUS	PERCENT	EXPECTED
14°C	118	3.3	106	3.0	4	.11	.10
16.5°C	123	2.2	118	2.2	3	.06	.05
25°C	138	1.4	247	2.6	5	.05	.04
30°C	261	5.3	312	6.0	14	.29	.32

If we assume that crossing over is as likely to occur in one part of a chromosome as in another, say in the first half of an arm as in the second, then the probability of a crossover in each half arm should be 1/4 at 30°C and the probability of doubles in each arm 1/16 or 6.3 percent, because strands that chiasmatized in the first half would still have a 1/4 probability of chiasmatizing again in the second, were there no interfering forces. The probability of simultaneous double crossovers in both arms would then be 1/256 or 0.39 percent. The last two columns of table 6 give the values for the obtained and expected percentages of simultaneous double crossovers in each arm. The numbers of flies are admittedly very small but we see that so far as this method goes there is virtually no interference at any temperature. No deductions concerning loop length can be made from that because we have no convenient point which divides the arm in halves. It can be

seen again that the values for the inversion-bearing chromosome are lower than those for the normal one.

It follows therefore that both arms of chromosome II are independent in crossing over when the arms as a whole are considered. We can check this statement by considering the cross given in table 8 (GRAUBARD 1932) as if performed for the regions *al-pr* and *pr-sp*, adjacent distances which cover approximately each arm. The following computations are obtained by treating the chromosome as if it carried only three genes—*al*, *pr* and *sp*. Hence known doubles between *al* and *pr* count as non-crossovers, and singles and triples as singles. The crossover values at 25°C are 35.3 percent and 42.0 percent respectively. The expected value of doubles is 14.8 percent and the actual figure obtained is 15.0 percent. Similarly at 30°C for the same cross the crossover value for the left arm is 41.4 percent and for the right 43.6 percent. The expected figure for doubles is 18.0 percent and that obtained is 18.2 percent. The same agreement is obtained for the normal chromosome. The cross reported in table 9 shows that taking the equivalent distances, coincidence for the two arms is $\frac{17.3}{17.6}$ at 25°C and $\frac{18.8}{18.8}$ at 30°C. These data show that no interference exists between the two arms.

We can carry our computation further and see whether by taking sections smaller than the whole arm, interference steps in. We can consider in the same manner the two adjacent regions *tx-pr* and *pr-L* in the normal chromosome (table 9). We are now dealing with central sections of chromatin the sum of which is a little over half the total length. The crossover value for the section to the left of the spindle fiber is 32.1 percent at 25°C and 35.7 percent at 30°C. For the section to the right of purple the values are 17.6 percent and 20.5 percent respectively. Coincidence at 25°C is $\frac{5.6}{5.6}$ and at 30°C $\frac{7.9}{7.3}$. Hence again no interference.

One more calculation is of interest. If we consider the distances *al-cn* and *cn-sp* in the normal chromosome, we have one section containing a spindle fiber attachment and a second one without a fiber connection. At 25°C the crossover values for *al-cn* and *cn-sp* are 41.7 percent and 42 percent and at 30°C 43.7 percent and 42.3 percent respectively. Coincidence at 25°C is $\frac{17.2}{17.5}$ and at 30°C $\frac{18.2}{18.4}$. We can take similar regions in the inversion-bearing chromosome, for example, *al-L* and *L-sp*. At 25°C the crossover values are 39.2 percent and 35.7 percent and coincidence is $\frac{13.3}{14.0}$. At 30°C the crossover values are 45.9 percent and 36.0 percent and coincidence $= \frac{16.7}{16.5}$.

These data together with those given in table 6 seem to show that if we take sufficiently large, adjacent regions there is no interference. They also show that the presence of a spindle fiber introduces no modifications. Coincidence in a V-shaped chromosome such as the second with a spindle fiber attachment in the middle seems to be dependent upon the lengths of regions rather than the spindle fiber mechanism.

However, the presence of the spindle fiber does seem to exert some influence. Two small adjacent regions on either side of it, although never

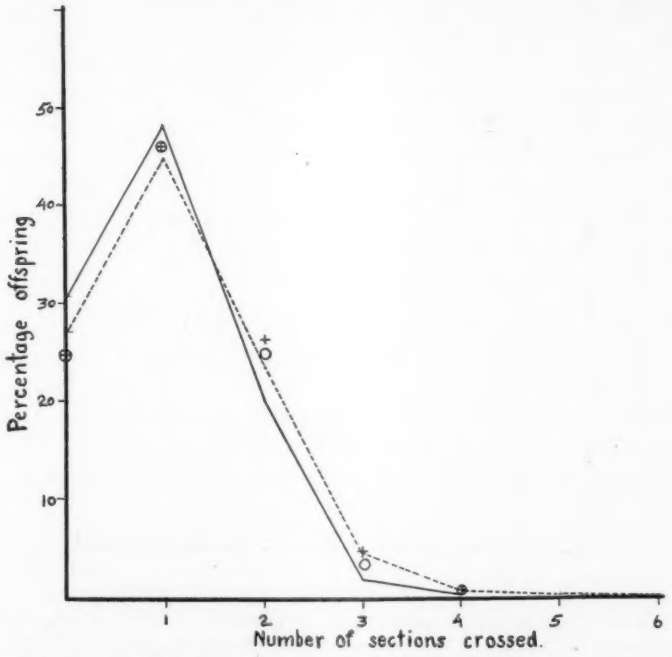

FIGURE 2.—Temperature effect on the frequency distribution of crossovers in the normal second chromosome of *Drosophila melanogaster*. Smooth line joins points obtained at 25° C, broken line at 30° C. Corresponding values at 14° C and 16° C are marked by 0 and + respectively.

showing a coincidence of one, show less interference than equivalent adjacent sections situated in one arm. Also regions lying very close to the point of spindle fiber attachment show an increase in their crossover values at the effective temperatures.

As shown in figures 2 and 3 the effective temperatures always diminish the non-crossover and as a rule the single crossover classes at the expense of an invariable increase in the number of multiple crossovers.

The effect of temperature seems to be limited to raising the probability

of crossing over in one section or another. In the case of chromosome II the effective temperatures operate so as to raise the basic crossover probability for small sections adjacent to the point of spindle fiber attachment. There is however no measurable influence on coincidence. What occurs is that due to the effective temperatures the probability of obtaining a genetic crossover in the regions adjacent to the spindle fiber is raised but

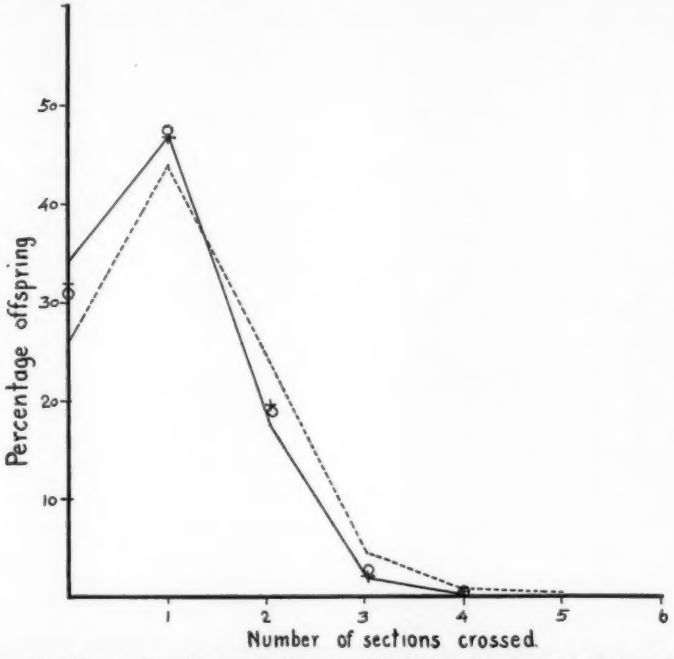

FIGURE 3.—Temperature effect on the frequency distribution of crossovers in the inversion-bearing second chromosome of *Drosophila melanogaster*. Smooth line joins points obtained at 25° C, broken line at 30° C. Corresponding values at 14° C and 16.5° C are marked by 0 and + respectively.

the probabilities for the further sections remain unaffected. The independence of the two sections still remains unaltered and the actual number of doubles is increased by exactly as much as should be expected on the basis of an increased frequency in one region only. This is demonstrated by the fact that the expected value, which is the product of the raised probability and the old probability for the unaffected section, is always in agreement with the figure actually obtained.

On the basis of DARLINGTON'S theory the number of chiasmata at 30°C should therefore be higher in the regions near the spindle fiber yet identical

for all temperatures in more distal sections, and the number of double chiasmata in each arm should be increased only in proportion to the rise in frequency of the central sections.

Whatever theory of crossing over is assumed the genetic evidence presented here seems to indicate that crossing over is so to speak, a property of a section of chromatin. For a given section there is a definite frequency of chiasmatization with a determined frequency of genetic crossing over. According to DARLINGTON'S theory the ratio of chiasmatization to genetic crossovers is 2:1. By observing the frequency of chiasmatization that theory can be tested. If we take a section of the proper length, we get independence of crossing over between one section and another. It should therefore follow that crossing over is not a central mechanism for chromosome II but a local one, and that one half of it would be equally independent of the other half in crossing over even were there no spindle fiber dividing them.

Similar results showing the absence of interference in sufficiently large sections, such as the two arms of chromosome II, are obtained if the data are calculated on the basis of crossings over instead of crossover flies, that is, when a double crossover is counted as two crossings, a triple as three, et cetera. We then find that the proportions of crossings over in the left arm are 44 percent at 25°C and 50 percent at 30°C. The values for the right arm are 49 percent at 25°C and 56 percent at 30°C. The percentages of crossings involved in the simultaneous crosses as calculated from the data are 40 percent and 52 percent at the two respective temperatures. The values expected on an independent basis are 42 percent and 56 percent.

The average percentage of crossings over in each arm of chromosome II is 46 percent, or 0.46 crossing per fly at 25°C. For the total chromosome it is 93 percent or 0.93 crossing per fly. For the whole X chromosome (BRIDGES and OLBRYCHT 1926) the respective value is 0.62 crossing per fly. Although the correlation between percentage crossings per fly is in perfect agreement with the lengths of chromatin as measured for the X, one arm and the entire chromosome II, it is doubtful whether such a comparison is fruitful. There is no reason to believe that equivalent lengths of chromatin have identical crossing over possibilities in different chromosomes.

It was noticed before (GRAUBARD 1932) that when small non-adjacent sections situated far apart are considered, as for example *al-dp* and *vg-L* or *al-dp* and *L-sp*, interference is always operating. This is not quite correct if we consider that in computing coincidence between non-adjacent regions, we include cases which show intermediate crossing over. These latter are bound to introduce errors since when they are involved we no

longer study the interaction between the two regions selected for observation but introduce disturbances known to take place.

To obviate this complication we can use only those data in table 9 (GRAUBARD 1932) which are relevant and disregard the others. In computing interference between *al-dp* (section 1) and *L-sp* (section 7) we must consider only four classes of flies: Non-crossovers, crossovers in region 1, crossovers in 7, double (1–7) crossovers. The total of the flies consists of the sum of these classes. If this procedure is followed we get the following coincidence values.

TABLE 7

Corrected coincidence values for various regions.

DESCRIPTION OF SECTIONS	SECTIONS	COINCIDENCE VALUES 25°C	30°C
Far apart. In different arms	1–7	.9	1.1
	2–5	.9	1.0
Central in one arm, extreme in the other	3–7	1.0	.9
	1–4	1.0	1.0
Central in one arm, distal in the other	3–5	1.1	1.0
	4–2	.8	1.0
Both sections in the same arm	4–7	.7	.8
	1–3	.5	.9
Both adjacent to the spindle fiber	3–4	.7	.5

We see from these figures that there is no interference between regions sufficiently far apart. The suggestion may therefore be made that the terms loop and interference are not applicable to sections further apart than the maximum loop length which is capable of exerting interference. The picture of a loop was postulated to account for interference. The concept of interference presumes a tendency to form a loop after one crossing has occurred. Hence when a crossover occurs in region 1 and another one in region 7 there is no reason to consider that a loop in the above sense. They are two events occurring simultaneously and apparently independently. It stands to reason that when crossings take place in regions adjacent to either one of the two, they will exert an influence. For this reason they are neglected in the above computation because only the two sections must be isolated for study if we wish to find the influence of a cross in one upon that in the other.

The fact that the same values are obtained at 30°C means that temperature does not modify that relationship. It is also seen that sections in the same arm always show interference and that two small sections on either

side of the spindle fiber show higher coincidence than two larger, adjacent sections in one arm.

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CONCLUSIONS

1. On the basis of DARLINGTON'S theory of crossing over there must be on the average at least one chiasmatization in each arm of the V-shaped second chromosome at 30°C.

2. The effective high and low temperatures decrease the percentage of non-crossovers and increase the frequency of multiple crossing over.

3. The effective temperatures raise the probability for getting a crossover in the small sections adjacent to the spindle fiber. They exert no influence on coincidence. The rise in multiple crossovers is due to the increase in the frequency of crossovers in the sections adjacent to the spindle fiber. The resulting increase in multiples is in good agreement with the magnitude expected on the basis of the probability values.

4. If large enough sections are considered, no interference obtains in chromosome II. Crossing over in one arm is independent of that in the other arm. No interference exists for any adjacent regions of large enough lengths regardless of whether they contain the spindle fiber toward the center or at extreme ends. The same independence holds true for non-adjacent sections sufficiently wide apart, provided we consider only flies relevant to the relationship under examination. For two sections situated in the same arm interference obtains.

5. If a section of chromatin of a certain length has a given probability of producing a crossover, then the term interference cannot be applicable to two crossovers further apart than the maximum interference-exerting, loop size. They will be two independent events occurring simultaneously. Events in the intervening regions may influence one section or the other and they must be accounted for.

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